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**Oyelere**(10) **Patent No.:** **US 8,188,054 B2**  
(45) **Date of Patent:** **May 29, 2012**(54) **NON-PEPTIDE MACROCYCLIC HISTONE DEACETYLASE (HDAC) INHIBITORS AND METHODS OF MAKING AND USING THEREOF**(75) Inventor: **Adegboyega Oyelere**, Marietta, GA (US)(73) Assignee: **Georgia Tech Research Corporation**, Atlanta, GA (US)

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(60) Provisional application No. 60/947,036, filed on Jun. 29, 2007.

(51) **Int. Cl.****A61K 31/70** (2006.01)**C07H 17/08** (2006.01)(52) **U.S. Cl.** ..... **514/29; 536/7.4**(58) **Field of Classification Search** ..... **536/7.4**  
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

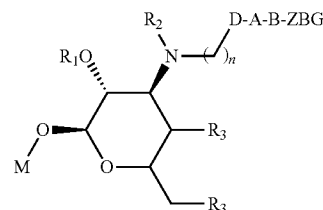
3,725,385 A	4/1973	Freiberg
5,631,355 A	5/1997	Asaka
6,054,435 A	4/2000	Or
6,579,986 B2	6/2003	Allen
7,091,187 B2	8/2006	Mercep
7,335,753 B2	2/2008	Wang
2004/0097434 A1	5/2004	Mercep
2005/0197334 A1*	9/2005	Wang et al. .... 514/227.5
2007/0149463 A1	6/2007	Oyelere

**OTHER PUBLICATIONS**Grozinger and Schreiber, "Deacetylase enzymes: biological functions and the use of small-molecule inhibitors.", *Chem. Biol.*, 9(1):3-16 (2002).Haggarty, et al., "Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation.", *Proc. Natl. Acad. Sci. USA*, 100(8):4389-4394 (2003).Kashimura, et al., "The synthesis and antibacterial activity of tetracyclic macrolides.", *J. Antibiot.*, 56(12):1062-1065 (2003).Kelly, et al., "Histone deacetylase inhibitors: from target to clinical trials.", *Expert. Opin. Invest. Drugs*, 11(12):1695-1713 (2002).Meinke, et al., "Histone deacetylase: a target for antiproliferative and antiprotozoal agents.", *Curr. Med. Chem.*, 8(2):211-235 (2001).Miller, "Histone deacetylase inhibitors.", *J. Med. Chem.*, 46(24):5097-5116 (2003).Monneret, "Histone deacetylase inhibitors", *Eur. J. of Med. Chem.*, 40(1):1-13 (2005).Morimoto, et al., "Chemical modification of erythromycins. II. Synthesis and antibacterial activity of O-alkyl derivatives of erythromycin A.", *J. Antibiot.*, 43 (3):286-294 (1990).Rosato and Grant, "Histone deacetylase inhibitors in clinical development", *Expert Opin. Invest. Drugs*, 13(1):21-38 (2004).Wong, et al., "Structural biasing elements for in-cell histone deacetylase paralog selectivity.", *J. Am. Chem. Soc.*, 125(19):5586-5587 (2003).

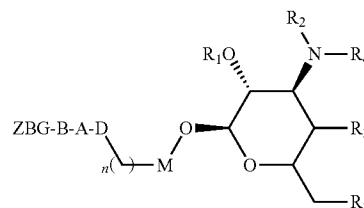
\* cited by examiner

*Primary Examiner* — Elli Peshev(74) *Attorney, Agent, or Firm* — Pabst Patent Group LLP(57) **ABSTRACT**

Compounds of Formula I or II, and methods of making and using thereof, are described herein.



I



II

M represents a macrolide subunit,

n is a C<sub>1-6</sub> group, optionally containing one or more heteroatoms,

D is an alkyl or aryl group,

A is a linking group connected to D,

B is an alkyl, alkylaryl or alkylheteroaryl spacer group,

ZBG is a Zinc Binding Group,

R<sub>1</sub>, R<sub>2</sub> and R<sub>4</sub> are independently selected from hydrogen, a C<sub>1-6</sub> alkyl group, a C<sub>2-6</sub> alkenyl group, a C<sub>2-6</sub> alkynyl group, a C<sub>1-6</sub> alkanooate group, a C<sub>2-6</sub> carbamate group, a C<sub>2-6</sub> carbonate group, a C<sub>2-6</sub> thiocarbamate group,R<sub>3</sub> is hydrogen or —OR<sub>5</sub>,R<sub>5</sub> is selected from a group consisting of Hydrogen, a C<sub>1-6</sub> alkyl hgroup, a C<sub>2-6</sub> alkenyl group, a C<sub>2-6</sub> alkynyl group, C<sub>1-6</sub> alkanooate group, C<sub>2-6</sub> carbamate group, C<sub>2-6</sub> carbonate group, C<sub>2-6</sub> thiocarbamate group,**26 Claims, No Drawings**

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# NON-PEPTIDE MACROCYCLIC HISTONE DEACETYLASE (HDAC) INHIBITORS AND METHODS OF MAKING AND USING THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of PCT/US2008/068787 filed Jun. 30, 2008, which claims priority to U.S. Ser. No. 60/947,036 filed Jun. 29, 2007.

## FIELD OF THE INVENTION

The present invention generally relates to non-peptide macrocyclic histone deacetylase (HDAC) inhibitors and methods of making and using thereof.

## BACKGROUND OF THE INVENTION

Histone deacetylases (HDACs) and histone acetyltransferases (HATS) are two functionally opposing enzymes which tightly regulate chromatin structure and function by maintaining the equilibrium between the acetylated- and deacetylated-states of nucleosomal histones. Aberrations in intracellular histone acetylation-deacetylation equilibrium have been linked to the repression of a subset of genes resulting in excessive proliferation and are implicated in a number of malignant diseases (Jenuwein, T.; Allis, C. D., *Science* 293, 1074-1080 (2001); Marks, P.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, T.; Kelly, W. K., *Nat. Rev. Cancer*, 1, 194-202 (2001)). HDACs function as part of multiprotein complexes that catalyze the removal of acetyl groups from the  $\epsilon$ -amino groups of specific lysine residues located near the N-termini of nucleosomal core histones (Grozinger, C. M.; Schreiber, S. L., *Chem. Biol.* 9, 3-16 (2002)). HDAC-catalyzed deacetylation results in positively charged, hypoacetylated histones which bind tightly to the phosphate backbone of DNA, thus inducing gene-specific repression of transcription. Inhibition of HDAC function results in the weakening of the bond between histones and DNA, thus increasing DNA accessibility and gene transcription.

Eighteen distinct human HDACs have been identified to date. They are classified into three major families based on their homology to three *Saccharomyces cerevisiae* HDACs: RPD3, HDAC1, and SIR2. Class I includes HDACs 1, 2, 3 and 8. Class II includes HDACs 4, 5, 6, 7, 9, 10 and 11. The third class of HDACs is the sirtuins, which are homologically distinct from all the currently known HDACs. HDAC inhibition by small molecules has been observed for the natural product (R)-trichostatin A which induced cell differentiation of murine erythroleukemia cells and hyperacetylation of histone proteins at nanomolar concentrations. Suberoylanilide hydroxamic acid (SAHA) has also been identified as a HDAC inhibitor.

Inhibition of HDACs is an emerging therapeutic strategy in cancer therapy. HDAC inhibitors have demonstrated ability to arrest proliferation of nearly all transformed cell types, including epithelial (melanoma, lung, breast, pancreas, ovary, prostate, colon and bladder) and hematological (lymphoma, leukemia and multiple myeloma) tumors (Kelly, W. K.; O'Connor, O. A.; Marks, P. A., *Expert Opin. Investig. Drugs*, 11, 1695-1713 (2002)). Additionally, HDAC inhibitors have demonstrated other biological activity including anti-inflammatory, anti-arthritis, anti-infective, anti-malarial, cytoprotective, neuroprotective, chemopreventive and/or cognitive enhancing effects.

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All HDAC inhibitors so far reported typically fit a three-motif pharmacophoric model namely, a zinc-binding group (ZBG), a hydrophobic linker and a recognition cap-group (Miller, T. A.; Witter, D. J.; Belvedere, S., *J. Med. Chem.*, 46, 5097-5116 (2003)). Structural modifications of the ZBG yielding hydroxamate isosteres such as benzamide,  $\alpha$ -ketoesters, electrophilic ketones, mercaptoamide and phosphonates have been reported. The cap-group may present opportunities to discover more potent and/or selective HDAC inhibitors. Toward this end, recent work by Schreiber and co-workers has led to the identification of cap group-modified agents that display differential inhibition against specific HDAC sub-types (Wong, J.; Hong, R.; Schreiber, S., *J. Am. Chem. Soc.* 125, 5586-5587 (2003); Haggarty, S. J.; Koeller, K. M.; Wong, J. C.; Grozinger, C. M.; Schreiber, S. L., *Proc. Natl. Acad. Sci. USA*, 100, 4389-4394 (2003)).

Cyclic-peptide moieties are the most complex of all HDAC inhibitor cap-groups and present an opportunity for the modulation of the biological activities of HDAC inhibitors. The macrocycle group is made up of hydrophobic amino acids and the prominent difference among the members of this class is in the amino acid side-chain substitution on the ring. Mechanistically, cyclic-peptide HDAC inhibitors can be divided into two classes: (i) reversible HDAC inhibitors and (ii) irreversible HDAC inhibitors, due to the alkylative modification of HDAC enzyme by the epoxy-ketone moiety on their side-chain. HDAC inhibitory activity and selectivity can vary significantly by changing the side-chain of each amino acid and/or the pattern of the combination of amino acid chirality.

Although cyclic-peptide HDAC inhibitors may possess potent HDAC inhibitory activity, their broad application in specific therapies, such as cancer therapy, currently remains largely unproven. The absence of clinically effective cyclic-peptide HDAC inhibitors may be in part due to development problems characteristic of large peptides, particularly poor oral bioavailability. In fact, the overall in vivo efficacy of cyclic-peptide HDAC inhibitors is complicated by their membrane penetration ability. HDAC inhibitory potency has been noted to increase with increase in the hydrophobicity of the macrocyclic ring (Meinke, P. T.; Liberator, P., *Curr. Med. Chem.*, 8, 211-235 (2001)). Unfortunately, SAR studies for this class of compounds have been impaired largely because most macrocyclic HDAC inhibitors known to date contain peptide macrocycles. In addition to retaining the pharmacologically disadvantaged peptidyl-backbone, they offer only limited opportunity for side-chain modifications.

To date, several other structurally distinct small molecule HDAC inhibitors have been reported including hydroxamates, benzamides, short-chain fatty acids, electrophilic ketones and cyclic-peptides (Miller, T. A.; Witter, D. J.; Belvedere, S., *J. Med. Chem.* 46, 5097-5116 (2003); Rosato, R. R.; Grant, S., *Expert Opin. Invest. Drugs*, 13, 21-38 (2004); Monneret, C., *Eur. J. of Med. Chem.*, 40, 1-13 (2005); Yoo, C. B.; Jones, P. A., *Nature Reviews Drug Discovery*, 5, 37-50 (2006)). Most of these agents have been shown to non-selectively inhibit the deacetylase activity of class I/II HDAC enzymes. The HDAC inhibitor SAHA has been approved by the FDA for the treatment of cutaneous T cell lymphoma. However, a large number of the identified HDAC inhibitors have elicited only limited in vivo antitumor activities and have not progressed beyond preclinical characterizations.

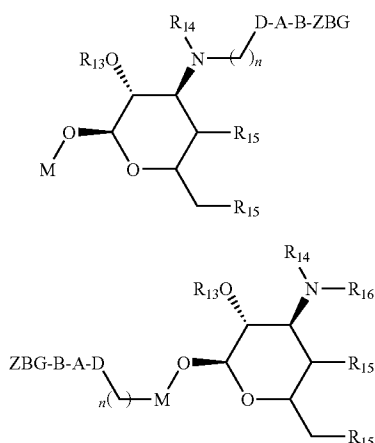
Therefore, there is a need to develop new HDAC inhibitors with improved efficacy, and better pharmacokinetic properties for use as therapeutic agents, such as anti-cancer agents and anti-parasitic agents.

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It is therefore an object of the invention to provide non-peptide macrocyclic HDAC inhibitors having improved efficacy and methods of making and using thereof.

### SUMMARY OF THE INVENTION

Compounds of Formula I and II, and methods of making and using thereof, are described herein.



wherein M represents a macrolide subunit,

n is a C<sub>1-6</sub> group, optionally containing one or more heteroatoms, wherein the carbon atoms and/or heteroatoms are in a linear and/or cyclic arrangement,

D is a substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl group,

A is a linking group connected to D,

B is an alkyl, heteroalkyl, alkylaryl or alkylheteroaryl spacer group,

ZBG is a Zinc Binding Group,

R<sub>13</sub> is selected from hydrogen, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group,

R<sub>14</sub> and R<sub>16</sub> is selected from hydrogen, hydroxyl, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group,

R<sub>15</sub> is hydrogen or —OR<sub>17</sub>,

R<sub>17</sub> is selected from a group consisting of hydrogen, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group.

The macrolide subunit can be attached to the pyran moiety at multiple locations on the macrolide subunit. In one

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embodiment, the compound contains the macrolide M9, M27, M28, M29, or M30 in Table 2, z is 1, n is 1, D is an aryl group, such as a phenyl group, A is a 1,2,3-triazolyl group, B is an alkyl group having from 5-8 carbons, and ZBG is a hydroxamate group.

The compounds can be administered as the free acid or base, or as a pharmaceutically acceptable salt, prodrug, or solvate. The compounds can be formulated with a pharmaceutically acceptable carrier and, optionally one or more pharmaceutically acceptable excipients, for enteral, parenteral, or topical administration. The compounds can be formulated for immediate release and/or controlled release. Examples of controlled release formulations include sustained release, delayed release, pulsatile release, and combinations thereof.

The compounds may be useful as anti-cancer agents, anti-inflammatory agents, anti-infective agents, anti-parasitic agents, such as anti-malarial agents and antileishmanial agents, cytoprotective agents, chemopreventive agents, prokinetic agents, and/or cognitive enhancing agents. The presence of the macrolide group may allow for the targeted delivery of the HDAC inhibitor in view on the ability of macrolides to accumulate in specific tissues.

### DETAILED DESCRIPTION OF THE INVENTION

#### I. Definitions

“Macrolide”, as used herein, includes, but is not limited to, multi-member lactonic ring molecules, wherein “member” refers to the carbon atoms and heteroatoms in the ring, and “multi” is a number greater than about 10, preferably from 10 to about 20, more preferably 12-, 14-, 15-, 16-, 17- or 18-member lactonic rings. Suitable macrolides include, but are not limited to, azithromycin and its derivatives; clarithromycin and its derivatives; erythromycin and its derivatives; bridged bicyclic macrolides, such as EDP-420 and its derivatives; dirithromycin and its derivatives, 9-dihydro-9-deoxy-9a-aza-9a-homoerythromycin and its derivatives; HMR 3004 and its derivatives; HMR 3647 and its derivatives; HMR 3787 and its derivatives; josamycin and its derivatives; erythromycinylamine and its derivatives; ABT 773 and its derivatives; TE 802 and its derivatives; flurithromycin and its derivatives; tylosin and its derivatives; tilmicin and its derivatives; oleandomycin and its derivatives; desmicosin and its derivatives; CP-163505 and its derivatives; EDP-420 and its derivatives; roxithromycin and its derivatives; miocamycin and its derivatives; rokitamycin and derivatives thereof, such as ketolides (e.g., 3-ketone), lactams (e.g., 8a- or 9a-lactams) and derivatives lacking one or more sugar moieties.

“Aryl”, as used herein, refers to 5-12-membered, preferably 5-, 6- and 7-membered aromatic, heterocyclic, fused aromatic, fused heterocyclic, biaromatic, or biheterocyclic ring systems, optionally substituted, for example, by halogens, alkyl-, alkenyl-, and alkynyl-groups. Broadly defined, “Ar”, as used herein, includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as “aryl heterocycles” or “heteroaromatics”. The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxy, amino, nitro, sulphydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, alde-

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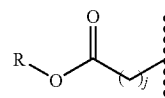
hyde, ester, heterocyclyl, aromatic or heteroaromatic moieties,  $-\text{CF}_3$ ,  $-\text{CN}$ , or the like. The term "Ar" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (i.e., "fused rings") wherein at least one of the rings aromatic, e.g., the other cyclic ring or rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocycles. Examples of heterocyclic ring include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolynyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolynyl, carbazolyl, 4aH carbazolyl, carbolynyl, chromanyl, chromenyl, cinnolynyl, decahydroquinolynyl, 2H,6H-1,5,2-dithiazynyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidynyl, imidazolynyl, imidazolyl, 1H-indazolyl, indolenyl, indolynyl, indolizynyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolynyl, isoindolyl, isoquinolynyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholynyl, naphthyridynyl, octahydroisoquinolynyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidynyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridynyl, phenanthrolinyl, phenazinyl, phenothiazynyl, phenoxathynyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidynyl, 4-piperidynyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidynyl, pyrazolynyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolynyl, quinolynyl, 4H-quinolizynyl, quinoxalynyl, quinuclidynyl, tetrahydrofuranyl, tetrahydroisoquinolynyl, tetrahydroquinolynyl, tetrazolyl, 6H-1,2,5-thiadiazynyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl.

"Zinc binding group" or "ZBG", as used herein, refers to a moiety of moieties capable of inhibiting the activity of zinc metalloenzymes including, but not limited to, HDAC and matrix metalloproteinase (MMP) activity. Suitable examples include, but are not limited to, hydroxamates, N-formyl hydroxylamine (or retro-hydroxamate), carboxylates, thiols, dithiols, trithiocarbonates, thioesters, benzamide, keto groups, mercaptoacetamides, 2-ketoamides, epoxides, epoxyketones, trifluoromethyl ketones, hydroxypyridinones, such as 2-hydroxy and 3-hydroxypyridinones, pyrones, hydroxylpyridinethiones, such as 3-hydroxy-2-methylpyridine-4-thione, and thiopyrones.

"Alkyl", as used herein, refers to the radical of saturated or unsaturated aliphatic groups, including straight-chain alkyl, alkenyl, or alkynyl groups, branched-chain alkyl, alkenyl, or alkynyl groups, cycloalkyl, cycloalkenyl, or cycloalkynyl (alicyclic) groups, alkyl substituted cycloalkyl, cycloalkenyl, or cycloalkynyl groups, and cycloalkyl substituted alkyl, alkenyl, or alkynyl groups. Unless otherwise indicated, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C1-C30 for straight chain, C3-C30 for branched chain), preferably 20 or fewer, more preferably 10 or fewer, most preferably 6 or less. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure. "Heteroalkyl", as used herein, refers to an alkyl group containing one or more heteroatoms, such as O, S, or N.

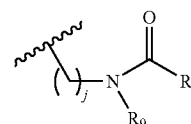
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"Alkoxy carbonyl", as used herein, refers to a substituent having the following chemical formula:



wherein R is a linear, branched, or cyclic alkyl group, wherein j is from about 1 to about 12.

"Alkoxy carbamido", as used herein, refers to a substituent having the following chemical formula:



wherein R<sub>8</sub> is alkoxy and R<sub>9</sub> is hydrogen, alkoxy-alkyl, or alkanoyl, and j is from about 1 to about 12.

"Alkylaryl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or hetero aromatic group).

"Heterocycle" or "heterocyclic", as used herein, refers to a cyclic radical attached via a ring carbon or nitrogen of a monocyclic or bicyclic ring containing 3-10 ring atoms, and preferably from 5-6 ring atoms, consisting of carbon and one to four heteroatoms each selected from the group consisting of non-peroxide oxygen, sulfur, and N(Y) wherein Y is absent or is H, O, (C<sub>1-4</sub>)alkyl, phenyl or benzyl, and optionally containing 1-3 double bonds and optionally substituted with one or more substituents. Examples of heterocyclic ring include, but are not limited to, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzoxazolynyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolynyl, carbazolyl, 4aH-carbazolyl, carbolynyl, chromanyl, chromenyl, cinnolynyl, decahydroquinolynyl, 2H,6H-1,5,2-dithiazynyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidynyl, imidazolynyl, imidazolyl, 1H-indazolyl, indolenyl, indolynyl, indolizynyl, indolyl, 3H-indolyl, isatinoyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolynyl, isoindolyl, isoquinolynyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholynyl, naphthyridynyl, octahydroisoquinolynyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidynyl, oxazolyl, oxindolyl, pyrimidinyl, phenanthridynyl, phenanthrolinyl, phenazinyl, phenothiazynyl, phenoxathynyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidynyl, 4-piperidynyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidynyl, pyrazolynyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolynyl, quinolynyl, 4H-quinolizynyl, quinoxalynyl, quinuclidynyl, tetrahydrofuranyl, tetrahydroisoquinolynyl, tetrahydroquinolynyl, tetrazolyl, 6H-1,2,5-thiadiazynyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl and xanthenyl.

"Heteroaryl", as used herein, refers to a monocycle aromatic ring containing five or six ring atoms consisting of carbon and 1, 2, 3, or 4 heteroatoms each selected from the

group consisting of non-peroxide oxygen, sulfur, and N(Y) where Y is absent or is H, O, (C<sub>1</sub>-C<sub>8</sub>) alkyl, phenyl or benzyl. Non-limiting examples of heteroaryl groups include furyl, imidazolyl, triazolyl, triazinyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl, (or its N-oxide), thienyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide), quinolyl (or its N-oxide) and the like. The term "heteroaryl" can include radicals of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a bent-derivative or one derived by fusing a propylene, trimethylene, or tetramethylene diradical thereto. Examples of heteroaryl can be furyl, imidazolyl, triazolyl, triazinyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, pyrrolyl, pyrazinyl, tetrazolyl, pyridyl (or its N-oxide), thienyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide), quinolyl (or its N-oxide), and the like.

"Halogen", as used herein, refers to fluorine, chlorine, bromine, or iodine.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The terms ortho, meta and para apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

"Substituted", as used herein, means that the functional group contains one or more substituents including, but not limited to, hydroxyl, halogen, nitro, aldehyde, ketone, carboxylic acid, ester, ether, amino, alkyl amino, dialkyl amino, amide, thiol, thioether, thione, sulfate, phosphate, and combinations thereof.

"Pharmaceutically acceptable salt", as used herein, refer to derivatives of the compounds defined by Formula I and II wherein the parent compound is modified by making acid or base salts thereof. Example of pharmaceutically acceptable salts include but are not limited to mineral or organic acid salts of basic residues such as amines; and alkali or organic salts of acidic residues such as carboxylic acids. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, and nitric acids; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pantoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, naphthalenesulfonic, methanesulfonic, ethane disulfonic, oxalic, and isethionic salts.

The pharmaceutically acceptable salts of the compounds can be synthesized from the parent compound, which contains a basic or acidic moiety, by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, non-aqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 20th ed., Lippincott Williams & Wilkins, Baltimore, Md., 2000, p. 704; and "Handbook of Pharmaceutical Salts: Properties, Selection, and Use," P. Heinrich Stahl and Camille G. Wermuth, Eds., Wiley-VCH, Weinheim, 2002.

As generally used herein "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

"Prodrug", as used herein, refers to a pharmacological substance (drug) which is administered in an inactive (or significantly less active) form. Once administered, the prodrug is metabolized in the body (in vivo) into the active compound.

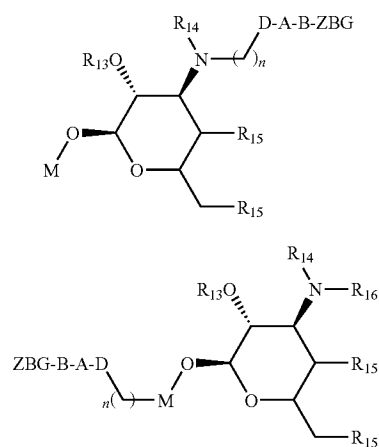
"Solvate", as used herein, refers to a compound which is formed by the interaction of molecules of a solute with molecules of a solvent.

"Reverse ester", as used herein, refers to the interchange of the positions of the oxygen and carbon groups in a series of structurally related compounds

"Reverse amide", as used herein, refers to the interchange of the positions of the nitrogen and carbon groups in a series of structurally related compounds.

## II. Compounds

Compounds of Formula I or II, and methods of making and using thereof, are described herein.



wherein M represents a macroide subunit, n is a C<sub>1-6</sub> group, optionally containing one or more heteroatoms, wherein the carbon atoms and/or heteroatoms are in a linear and/or cyclic arrangement,

D is a substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl group,

A is a linking group connected to D,

B is an alkyl, heteroalkyl, alkylaryl or alkylheteroaryl spacer group,

ZBG is a Zinc Binding Group,

R<sub>13</sub> is selected from hydrogen, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group,

R<sub>14</sub> and R<sub>15</sub> is selected from hydrogen, hydroxyl, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted

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tuted  $C_{2-6}$  alkynyl group, a substituted or unsubstituted  $C_{1-6}$  alkanate group, a substituted or unsubstituted  $C_{2-6}$  carbamate group, a substituted or unsubstituted  $C_{2-6}$  carbonate group, a substituted or unsubstituted  $C_{2-6}$  carbamate group, or a substituted or unsubstituted  $C_{2-6}$  thiocarbamate group,

$R_{15}$  is hydrogen or  $-OR_{17}$ ,

$R_{17}$  is selected from a group consisting of hydrogen, a substituted or unsubstituted  $C_{1-6}$  alkyl group, a substituted or unsubstituted  $C_{2-6}$  alkenyl group, a substituted or unsubstituted  $C_{2-6}$  alkynyl group, a substituted or unsubstituted  $C_{1-6}$  alkanate group, a substituted or unsubstituted  $C_{2-6}$  carbamate group, a substituted or unsubstituted  $C_{2-6}$  carbonate group, or a substituted or unsubstituted  $C_{2-6}$  thiocarbamate group.

Examples of the linking group A include, but are not limited to, amide, reverse amide, ester, reverse ester, alkoxyl, sulfanyl, sulfonyl, sulfonyl, sulfonamido, ketone,  $sp^3$  hybridized carbon,  $sp^2$  hybridized carbon,  $sp$  hybridized carbon, 5 or 6 membered heterocyclic rings including but not limiting to 1,2,3-triazolyl, 1,2,4-triazolyl, 1-tetrazolyl, 1-indolyl, 1-indazolyl, 2-isindolyl, 7-oxo-2-isindolyl, 1-piranyl, 3-isothiazolyl, 4-isothiazolyl and 5-isothiazolyl, 1,3,4-oxadiazole, 4-oxo-2-thiazolyl or 5-methyl-1,3,4-thiadiazol-2-yl, thiazoledione, 1,2,3,4-thiatriazole, 1,2,4-dithiazolone, pyridine, thiophene, furan, pyrazoline, pyrimidine, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 3-pyridazinyl, 4-pyridazinyl, 3-pyrazolyl, 2-quinolyl, 3-quinolyl, 1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 2-quinazolinyl, 4-quinazolinyl, 2-quinoxalyl, 1-phthalazinyl, 4-oxo-2-imidazolyl, 2-imidazolyl, 4-imidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 3-pyrazolyl, 4-pyrazolyl, 5-pyrazolyl, 2-oxazolyl, 4-oxazolyl, 4-oxo-2-oxazolyl, 5-oxazolyl, 4,5-dihydrooxazole, 1,2,3-oxathiole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 3-isothiazole, 4-isothiazole, 5-isothiazole, 2-indolyl, 3-indolyl, 3-indazolyl, 2-benzoxazolyl, 2-benzothiazolyl, 2-benzimidazolyl, 2-benzofuranyl, 3-benzofuranyl, benzoisothiazole, benzisoxazole, 2-furanyl, 3-furanyl, 2-thienyl, 3-thienyl, 2-pyrrolyl, 3-pyrrolyl, 3-isopyrrolyl, 4-isopyrrolyl, 5-isopyrrolyl, 1,2,3-oxathiazole-1-oxide, 1,2,4-oxadiazol-3-yl, 1,2,4-oxadiazol-5-yl, 5-oxo-1,2,4-oxadiazol-3-yl, 1,2,4-thiadiazol-3-yl, 1,2,4-thiadiazol-5-yl, 3-oxo-1,2,4-thiadiazol-5-yl, 1,3,4-thiadiazol-5-yl, 2-oxo-1,3,4-thiadiazol-5-yl, 1,2,4-triazol-3-yl, 1,2,4-triazol-5-yl, 1,2,3,4-tetrazol-5-yl, 5-oxazolyl, 1-pyrrolyl, 1-pyrazolyl. Each of these moieties may be substituted as appropriate.

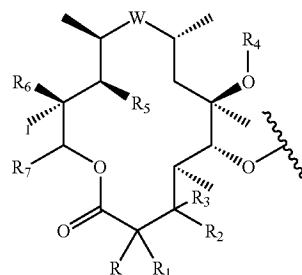
B is a substituted or unsubstituted alkyl, alkylaryl or alkylheteroaryl spacer group. Suitable alkyl spacer group chain length ranges from about  $C_4$  to about  $C_{12}$ , optionally substituted by one or more double and/or triple bonds. The total number of atoms in the alkylaryl and alkylheteroaryl groups is from about 6 to about 50, preferably from about 6 to about 30, more preferably from about 6 to about 20, most preferably from about 6 to about 10.

M is a macrolide subunit. Suitable macrolide subunits include, but are not limited to, multi-member lactonic ring molecules, wherein "member" refers to the carbon atoms or heteroatoms in the ring, and "multi" is a number greater than about 10, preferably from 10 to about 20, more preferably 12-, 14-, 15-, 16-, 17-, or 18-member lactonic rings. Exemplary macrolides include, but are not limited to, azithromycin and its derivatives; clarithromycin and its derivatives; erythromycin and its derivatives; bridged bicyclic macrolides, such as EDP-420 and its derivatives; dirithromycin and its derivatives, 9-dihydro-9-deoxy-9a-aza-9a-homoerythromycin and its derivatives; HMR 3004 and its derivatives, HMR

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3647 and its derivatives; HMR 3787 and its derivatives; josamycin and its derivatives; erythromycylamine and its derivatives; ABT 773 and its derivatives; TE 802 and its derivatives; flurithromycin and its derivatives; tylosin and its derivatives; tilmicosin and its derivatives; oleandomycin and its derivatives; desmicosin and its derivatives; CP-163505 and its derivatives; EDP-420 and its derivatives; roxithromycin and its derivatives; miocamycin and its derivatives; rokitamycin and derivatives thereof, such as ketolides (e.g., 3-ketone), lactams (e.g., 8a- or 9a-lactams) and derivatives lacking one or more sugar moieties.

In one embodiment, the macrolide M has the formula:



as described in U.S. Patent Application Publication No. 2007/0149463, which is incorporated herein by reference, wherein

W is selected from  $-C(O)-$ ,  $-C(=NOR_{11})-$ ,  $-CH(-OR_{11})-$ ,  $-NR_{11}CH_2-$ ,  $-CH_2NR_{11}-$ ,  $-CH(NR_{11}R_{11})-$ ,  $-C(=NNR_{11}R_{11})-$ ,  $-NR_{11}C(O)-$ ,  $-C(O)NR_{11}-$ , and  $-C(=NR_{11})-$ ;

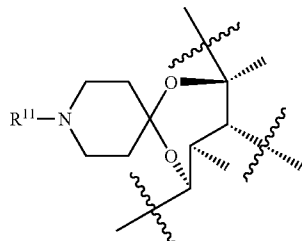
R is selected from the group consisting of H and  $C_{1-6}$  alkyl;

$R_1$  is selected from the group H, halogen,  $-NR_{11}R_{11}$ ,  $NR_{11}C(O)R_{11}$ ,  $-OR_{11}$ ,  $-OC(O)R_{11}$ ,  $-OC(O)OR_{11}$ ,  $-OC(O)NR_{11}R_{11}$ ,  $-OC_{1-6}alkyl-R_{12}$ ,  $-OC(O)C_{1-6}alkyl-R_{12}$ ,  $-OC(O)OC_{1-6}alkyl-R_{12}$ ,  $-OC(O)NR_{11}C_{1-6}alkyl-R_{12}$ ,  $C_{1-6}alkyl$ ,  $C_{1-6}alkenyl$ ,  $C_{1-6}alkynyl$ , optionally is substituted with one or more  $R_{12}$  groups;

$R_2$  is H;

$R_3$  is selected from H,  $-OR_{11}$ ,  $-OC_{1-6}alkyl-R_{12}$ ,  $-OC(O)R_{11}$ ,  $-OC(O)C_{1-6}alkyl-R_{12}$ ,  $-OC(O)OR_{11}$ ,  $-OC(O)OC_{1-6}alkyl-R_{12}$ ,  $-OC(O)NR_{11}R_{11}$ ,  $-OC(O)NR_{11}C_{1-6}alkyl-R_{12}$ ; alternatively,  $R_3$  is a pyran ring which can be substituted as defined above in Formulae I and II;

$R_4$  is selected from H,  $R_{11}$ ,  $-C(O)R_{11}$ ,  $-C(O)OR_{11}$ ,  $-C(O)NR_{11}R_{11}$ ,  $-C_{1-6}alkyl-T-R_{11}$ ,  $-C_{2-6}alkenyl-T-R_{11}$ , and  $C_{2-6}alkynyl-T-R_{11}$ ; alternatively  $R_3$  and  $R_4$  taken together form

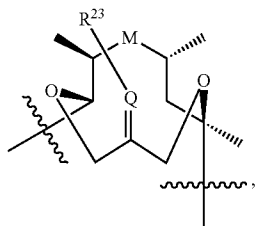


T is selected from  $-C(O)-$ ,  $-C(O)O-$ ,  $-C(O)NR_{11}-$ ,  $-C(=NR_{11})-$ ,  $-C(=NR_{11})O-$ ,  $-C(=NR_{11})NR_{11}-$ ,  $g) -OC(O)-$ ,  $-OC(O)O-$ ,  $-OC(O)NR_{11}-$ ,  $-NR_{11}C(O)O-$ ,  $-NR_{11}C(O)NR_{11}-$ ,  $-NR_{11}C(=NR_{11})NR_{11}-$ , and  $-S(O)_p-$ , wherein p 0-2;

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$R_5$  is selected from  $R_{11}$ ,  $-\text{OR}_{11}$ ,  $-\text{NR}_{11}\text{R}_{11}$ ,  $-\text{OC}_{1-6}$  alkyl- $\text{R}_{12}$ ,  $-\text{C}(\text{O})\text{R}_{11}$ ,  $-\text{C}(\text{O})\text{C}_{1-6}$  alkyl- $\text{R}_{12}$ ,  $-\text{OC}(\text{O})\text{R}_{11}$ ,  $-\text{OC}(\text{O})\text{G}_{1-6}$  alkyl- $\text{R}_{12}$ ,  $-\text{OC}(\text{O})\text{OR}_{11}$ ,  $-\text{OC}(\text{O})\text{OC}_{1-6}$  alkyl- $\text{R}_{12}$ ,  $-\text{OC}(\text{O})\text{NR}_{11}\text{R}_{11}$ ,  $-\text{OC}(\text{O})\text{NR}_{11}\text{C}_{1-6}$  alkyl- $\text{R}_{12}$ ,  $-\text{C}(\text{O})\text{C}_{2-6}$  alkenyl- $\text{R}_{12}$ , and  $-\text{C}(\text{O})\text{C}_{2-6}$  alkynyl- $\text{R}_{12}$ ;

alternatively,  $R_4$  and  $R_5$  taken together with the atoms to which they are bonded, form:

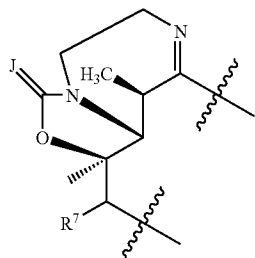


wherein, Q is CH or N, and  $R_{23}$  is  $-\text{OR}_{11}$  or  $\text{R}_{11}$ ;

$R_6$  is selected from  $-\text{OR}_{11}$ ,  $-\text{C}_{1-6}$  alkoxy- $\text{R}_{12}$ ,  $-\text{C}(\text{O})\text{R}_{11}$ ,  $-\text{OC}(\text{O})\text{R}_{11}$ ,  $-\text{OC}(\text{O})\text{OR}_{11}$ ,  $-\text{OC}(\text{O})\text{NR}_{11}\text{R}_{11}$ ,  $-\text{NR}_{11}\text{R}_{11}$ ;

alternatively,  $R_5$  and  $R_6$  taken together with the atoms to which they are attached form a 5-membered ring by attachment to each other through a linker selected from  $-\text{OC}(\text{R}_{12})_2\text{O}-$ ,  $-\text{OC}(\text{O})\text{O}-$ ,  $-\text{OC}(\text{O})\text{NR}_{11}-$ , d)  $-\text{NR}_{11}\text{C}(\text{O})\text{O}-$ ,  $-\text{OC}(\text{O})\text{NOR}_{11}-$ ,  $-\text{NOR}_{11}\text{C}(\text{O})\text{O}-$ ,  $-\text{OC}(\text{O})\text{NNR}_{11}-$ ,  $-\text{NNR}_{11}\text{R}_{11}\text{C}(\text{O})\text{O}-$ ,  $-\text{OC}(\text{O})\text{C}(\text{R}_{12})_2-$ ,  $-\text{C}(\text{R}_{12})_2\text{C}(\text{O})\text{O}-$ ,  $-\text{OC}(\text{S})\text{O}-$ ,  $-\text{OC}(\text{S})\text{NR}_{11}-$ ,  $-\text{NR}_{11}\text{C}(\text{S})\text{O}-$ , n)  $-\text{OC}(\text{S})\text{NOR}_{11}-$ ,  $-\text{NOR}_{11}\text{C}(\text{S})\text{O}-$ ,  $-\text{OC}(\text{S})\text{NNR}_{11}\text{R}_{11}-$ ,  $-\text{NNR}_{11}\text{R}_{11}\text{C}(\text{S})\text{O}-$ ,  $-\text{OC}(\text{S})\text{C}(\text{R}_{12})_2-$ ,  $-\text{C}(\text{R}_{12})_2\text{C}(\text{S})\text{O}-$ ;

alternatively, W,  $R_5$ , and  $R_6$  taken together with the atoms to which they are attached form:



wherein J is selected from the group consisting of O, S, and  $\text{NR}_{11}$ ;

$R_6$  is selected from H, unsubstituted or substituted  $\text{C}_{1-4}$  alkyl,  $\text{C}_{2-4}$  alkenyl, which can be further substituted with  $\text{C}_{1-2}$  alkyl or one or more halogens,  $\text{C}_{2-4}$  alkynyl, which can be further substituted with  $\text{C}_{1-2}$  alkyl or one or more halogens, aryl or heteroaryl, which can be further substituted with  $\text{C}_{1-2}$  alkyl or one or more halogens,  $-\text{C}(\text{O})\text{H}$ ,  $-\text{COOH}$ ,  $-\text{CN}$ ,  $-\text{COOR}_{11}$ ,  $-\text{C}(\text{O})\text{NR}_{11}\text{R}_{11}$ ,  $-\text{C}(\text{O})\text{R}_{11}$ ,  $-\text{C}(\text{O})\text{SR}_{11}$ ;

alternatively  $R_6$  and  $R_6$  taken together with the atom to which they are attached to form an epoxide, a carbonyl, an olefin, or a substituted olefin, or a  $\text{C}_3$ - $\text{C}_7$  carbocyclic, carbonate, or carbamate, wherein the nitrogen of the carbamate can be further substituted with a  $\text{C}_1$ - $\text{C}_6$  alkyl;

$R_7$  is selected from  $\text{C}_{1-6}$  alkyl,  $\text{C}_{2-6}$  alkenyl, and  $\text{C}_{2-6}$  alkynyl, optionally is substituted with one or more  $\text{R}_{12}$  groups;

## 12

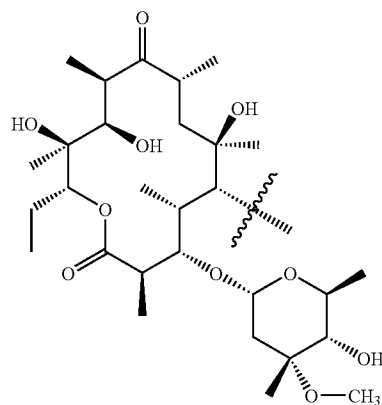
$\text{R}_{11}$ , for each occurrence, is selected from H,  $\text{C}_{1-6}$  alkyl,  $\text{C}_{2-6}$  alkenyl,  $\text{C}_{2-6}$  alkynyl,  $\text{C}_{6-10}$  saturated, unsaturated, or aromatic carbocycle, 3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from nitrogen, oxygen, and sulfur,  $-\text{C}(\text{O})-\text{C}_{1-6}$  alkyl,  $-\text{C}(\text{O})\text{C}_{2-6}$  alkenyl,  $-\text{C}(\text{O})-\text{C}_{2-6}$  alkynyl,  $-\text{C}(\text{O})-\text{C}_{6-10}$  saturated, unsaturated or aromatic carbocycle,  $-\text{C}(\text{O})$ -3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from nitrogen, oxygen, and sulfur,  $-\text{C}(\text{O})\text{O}-\text{C}_{1-6}$  alkyl,  $-\text{C}(\text{O})\text{O}-\text{C}_{2-6}$  alkenyl,  $-\text{C}(\text{O})\text{O}-\text{C}_{2-6}$  alkynyl,  $-\text{C}(\text{O})\text{O}-\text{C}_{6-10}$  saturated, unsaturated, or aromatic carbocycle,  $-\text{C}(\text{O})\text{O}$ -3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur, and  $-\text{C}(\text{O})\text{NR}_{13}\text{R}_{13}$ , optionally is substituted with one or more  $\text{R}_{12}$  groups,

$\text{R}_{12}$  is selected from  $\text{R}_{14}$ ,  $\text{C}_{1-8}$  alkyl,  $\text{C}_{2-8}$  alkenyl,  $\text{C}_{2-8}$  alkynyl,  $\text{C}_{3-12}$  saturated, unsaturated, or aromatic carbocycle, 3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from nitrogen, oxygen, and sulfur, optionally substituted with one or more substituents.

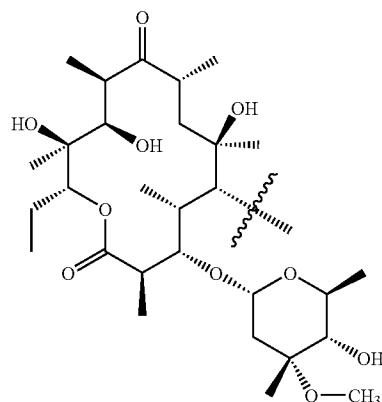
Exemplary macrolides are shown in Tables 1 and 2.

TABLE 1

Macrolide Subunits



M1

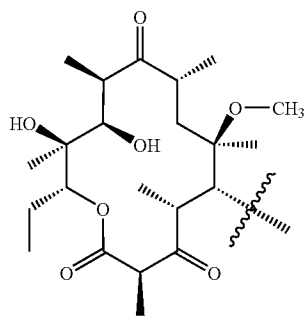


M2

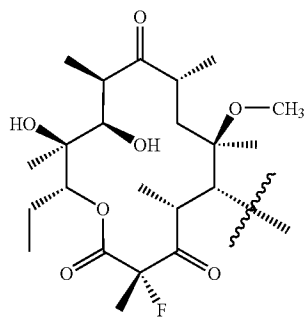
**13**

TABLE 1-continued

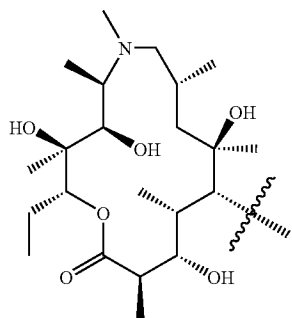
Macrolide Subunits



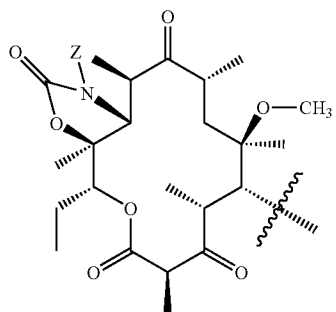
M3



M4



M5

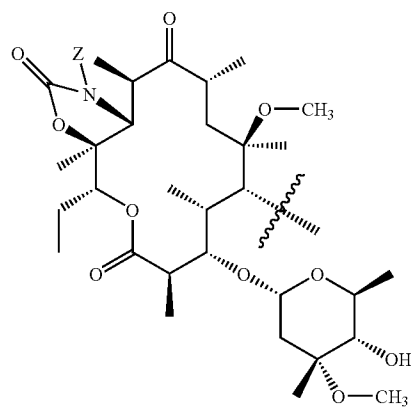


M6

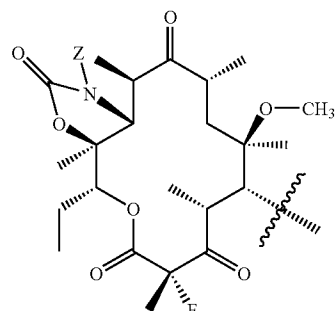
**14**

TABLE 1-continued

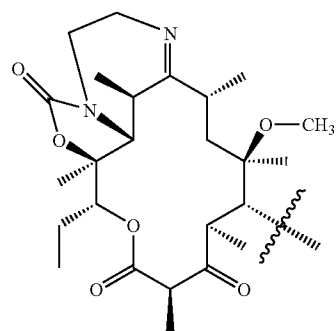
Macrolide Subunits



M7



M8



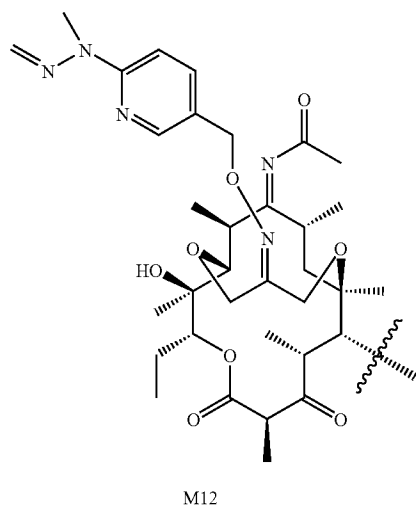
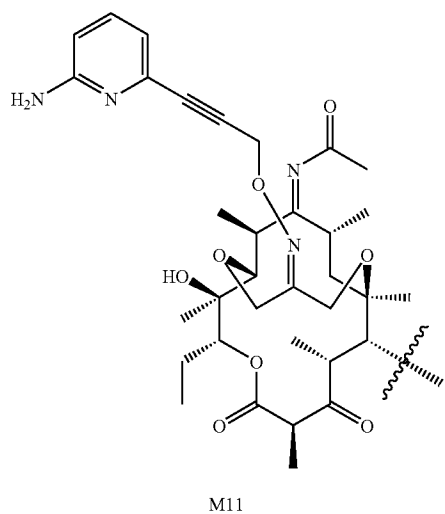
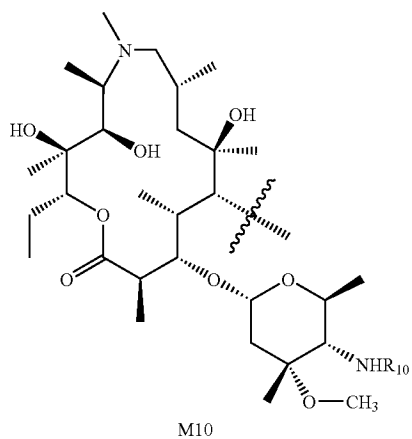
M9



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TABLE 1-continued

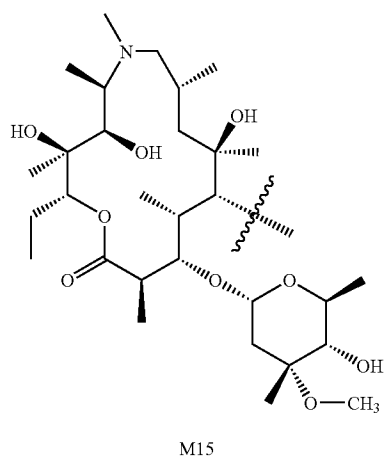
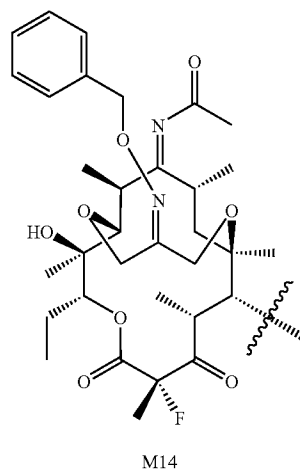
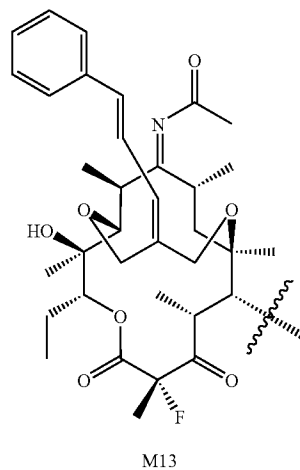
Macrolide Subunits



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TABLE 1-continued

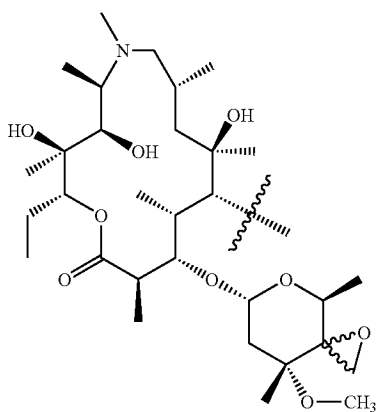
Macrolide Subunits



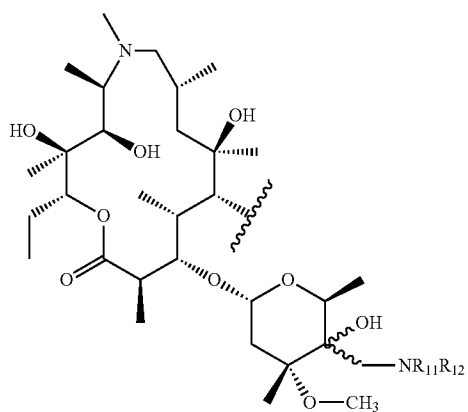
17

TABLE 1-continued

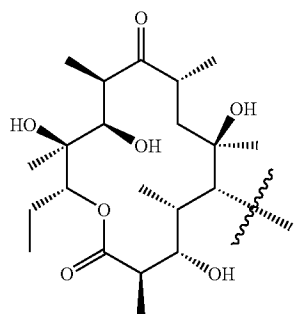
Macrolide Subunits



M16



M17



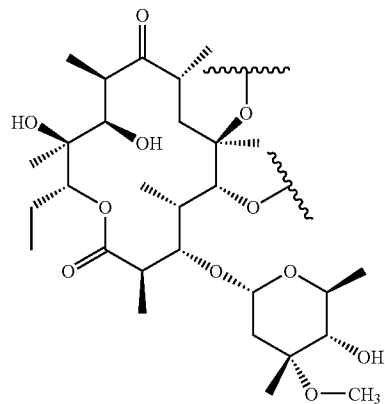
M18

18

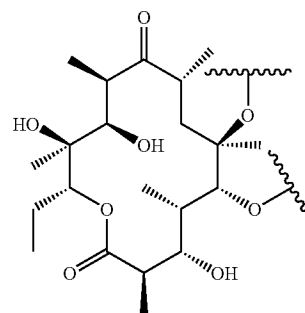
The pyran-ZBG group can be attached to macrolide at various points as shown in Table 2:

TABLE 2

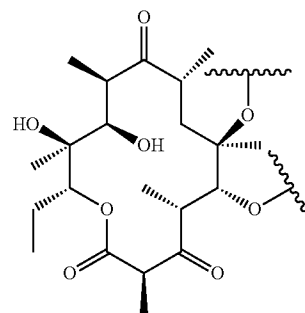
14-Membered Macrolides



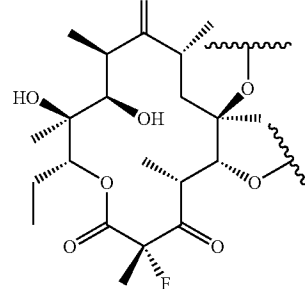
M19



M20



M21

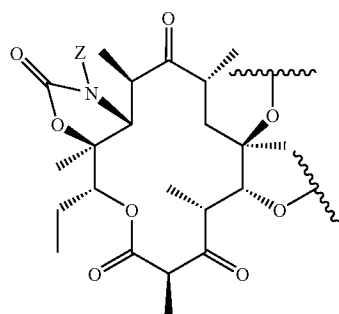


M22

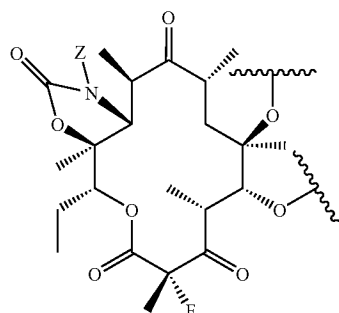
19

TABLE 2-continued

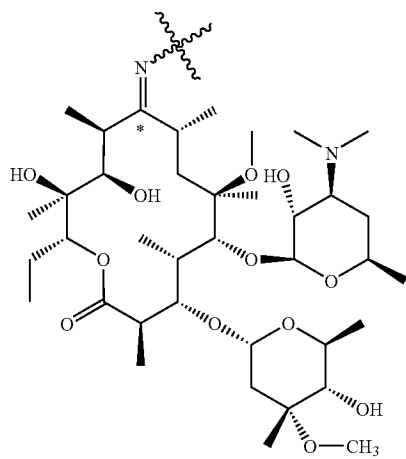
14-Membered Macrolides



M23



M24

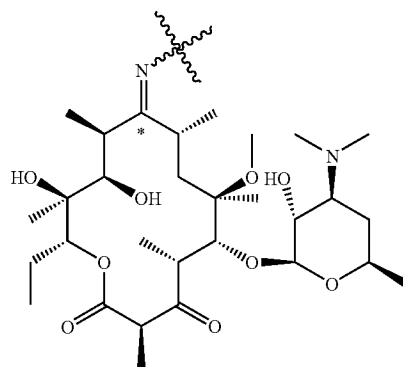


M25

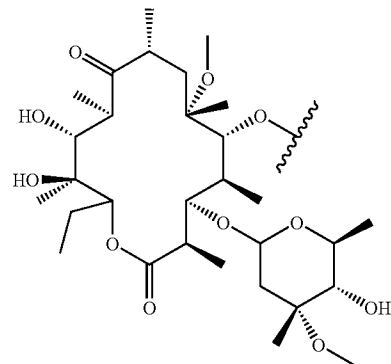
20

TABLE 2-continued

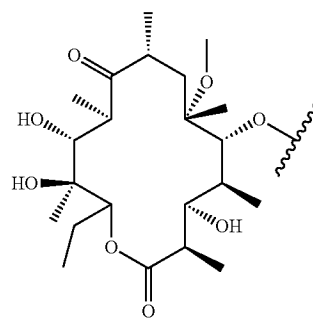
14-Membered Macrolides



M26



M27

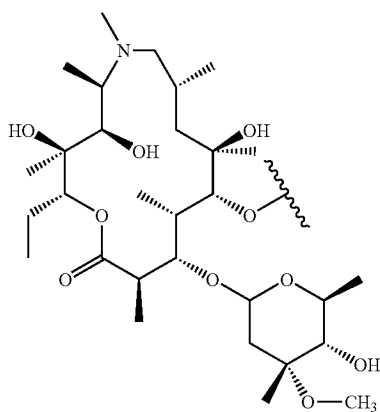


M28

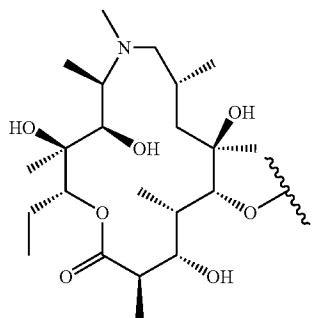
21

TABLE 2-continued

14-Membered Macrolides



M29



M30

In M25 and M26, the bond between the starred carbon and the nitrogen can be a single bond or a double bond. This is represented by a dashed line in the structures of M25 and M26.

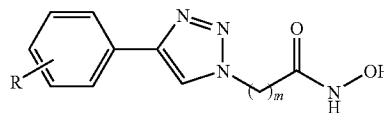
The compounds described herein may have one or more chiral centers and thus exist as one or more stereoisomers. Such stereoisomers can exist as a single enantiomer, a mixture of diastereomers or a racemic mixture.

As used herein, the term “stereoisomers” refers to compounds made up of the same atoms having the same bond order but having different three-dimensional arrangements of atoms which are not interchangeable. The three-dimensional structures are called configurations. As used herein, the term “enantiomers” refers to two stereoisomers which are non-superimposable mirror images of one another. As used herein, the term “optical isomer” is equivalent to the term “enantiomer”. As used herein the term “diastereomer” refers to two stereoisomers which are not mirror images but also not superimposable. The terms “racemate”, “racemic mixture” or “racemic modification” refer to a mixture of equal parts of enantiomers. The term “chiral center” refers to a carbon atom to which four different groups are attached. Choice of the appropriate chiral column, eluent, and conditions necessary to effect separation of the pair of enantiomers is well known

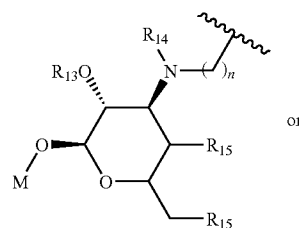
22

to one of ordinary skill in the art using standard techniques (see e.g. Jacques, J. et al., “Enantiomers, Racemates, and Resolutions”, John Wiley and Sons, Inc. 1981).

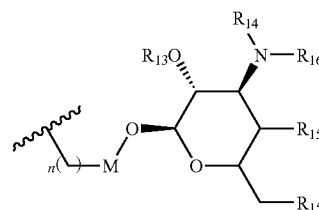
In one embodiment, the HDAC inhibitors having the formula:



wherein R is:



or

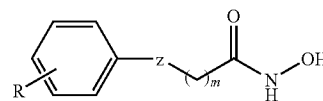


and m is an integer from 1-12, preferably from 1-10, more preferably from 4-10, most preferably from 5-9 inclusive. However, compounds where m is greater than 12 may be prepared and their activity determined as described in the examples.

R can be positioned ortho, meta, or para to the triazolyl group. In one embodiment, R is para to the triazolyl group. In another embodiment, R is meta to the triazolyl group.

The macrolide can be any suitable macrocyclic compound. In one embodiment, the macrolide is selected from M9 or M27-M30 in Table 2.

In another embodiment, the HDAC inhibitors have the following formula:



wherein R and M are as defined above and z is oxygen or —NCO—.

In still another embodiment, the HDAC inhibitor has one of the formulas above, wherein the zinc binding group is a pyridine thione, diamino benzene, or hydroxypyridone group.

Non-limiting examples of HDAC inhibitors of Formula I and II are shown in Table 3.

TABLE 3

Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
7	
8	
9	

### Non-peptide HDAC Inhibitors

COM- POUND NUMBER	STRUCTURE
10	
11	
12	
13	

TABLE 3-continued

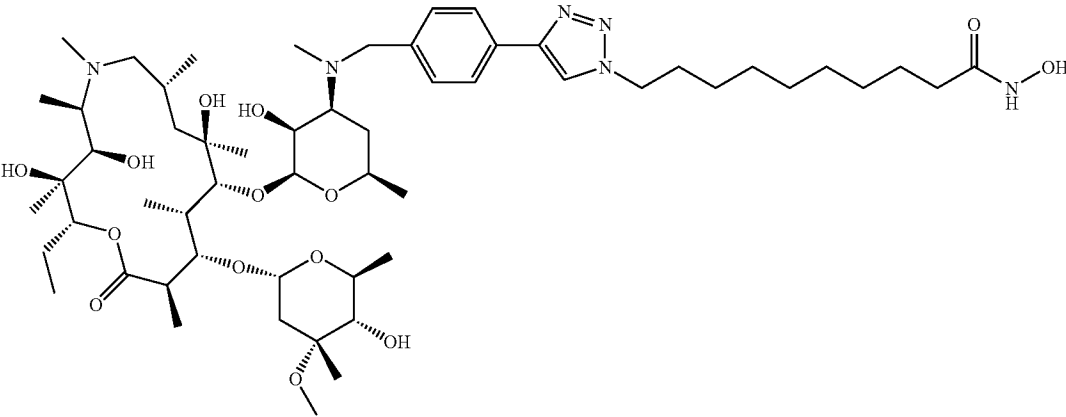
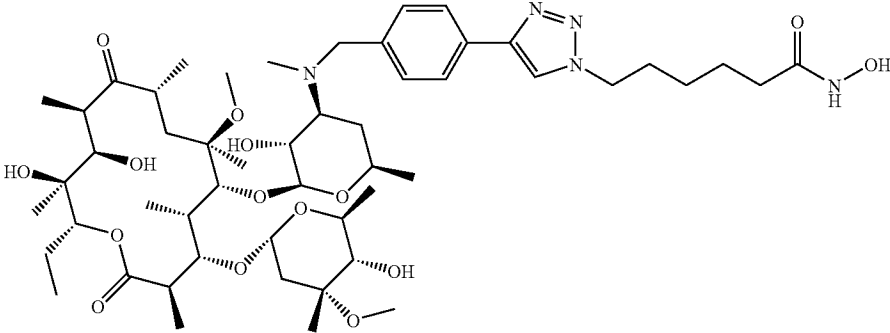
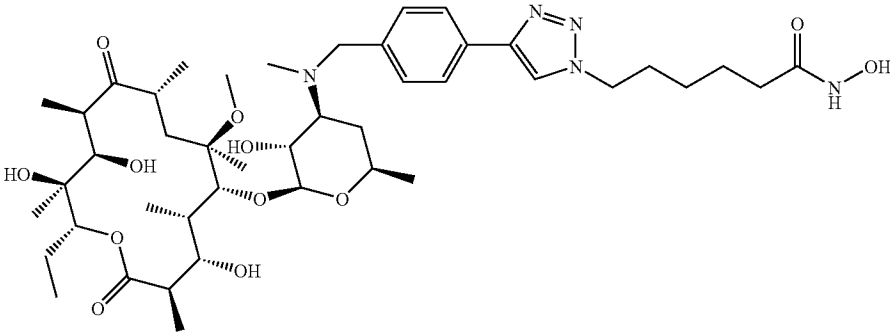
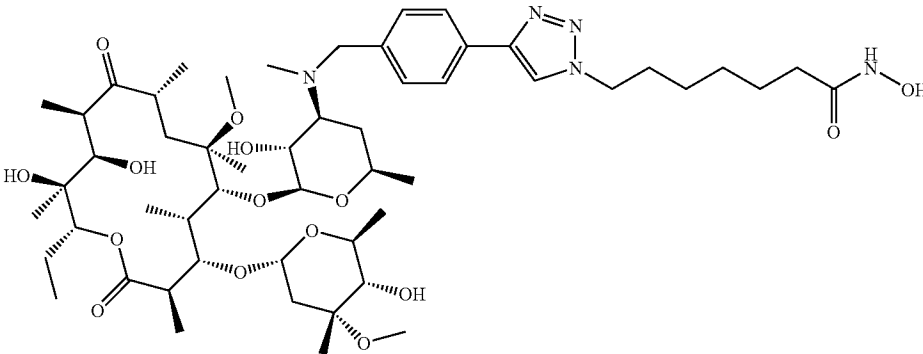
COM- POUND NUMBER	STRUCTURE
14	
23	
24	
25	

TABLE 3-continued

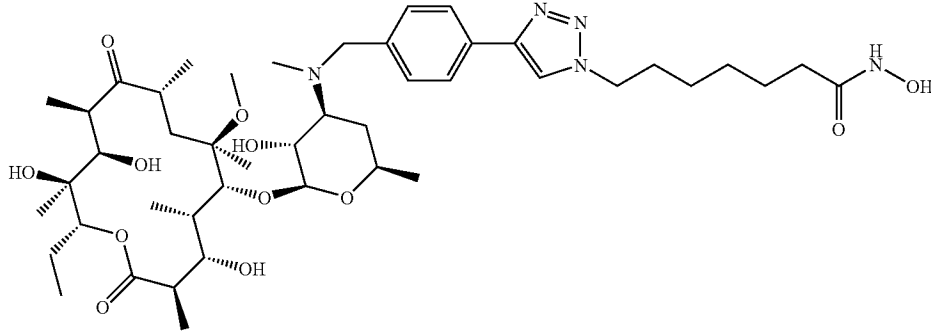
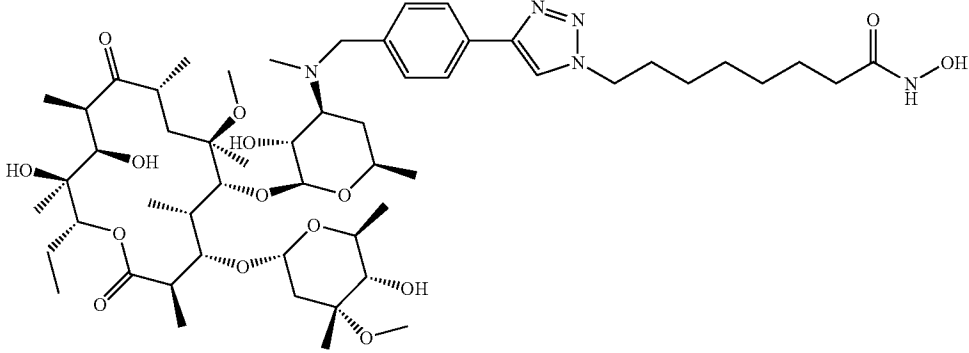
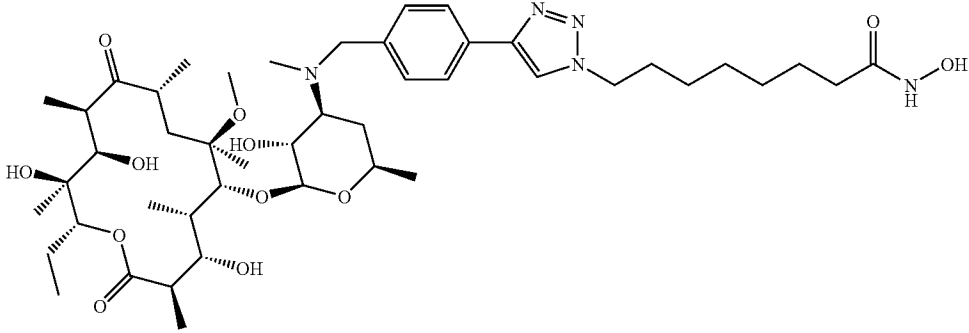
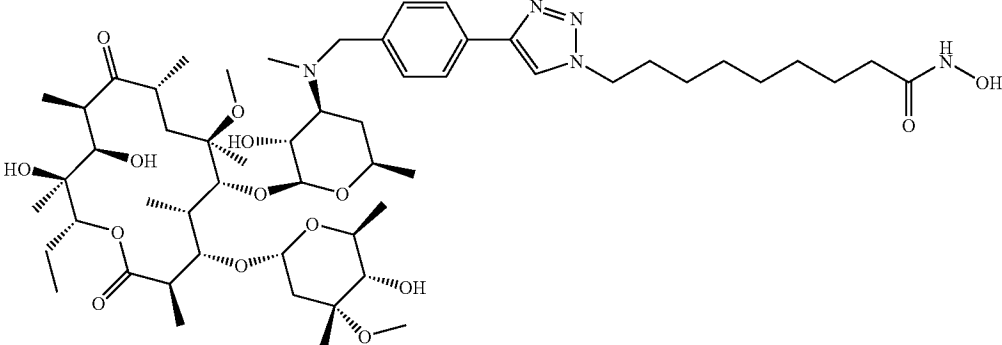
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
26	
27	
28	
29	



TABLE 3-continued

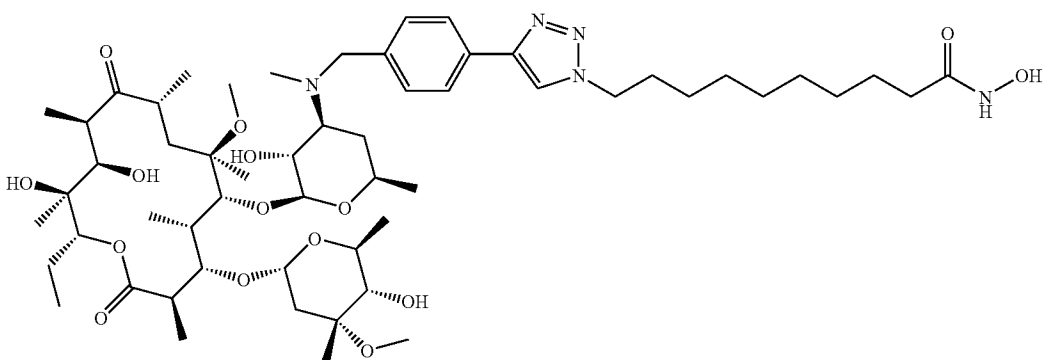
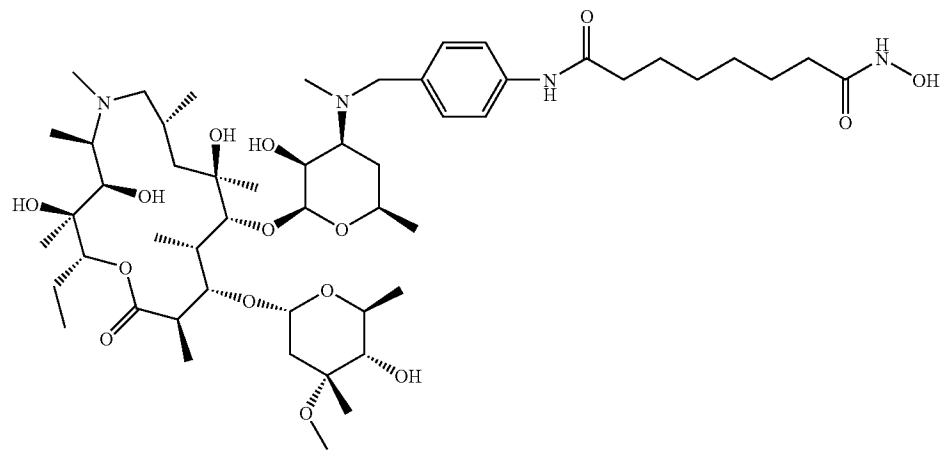
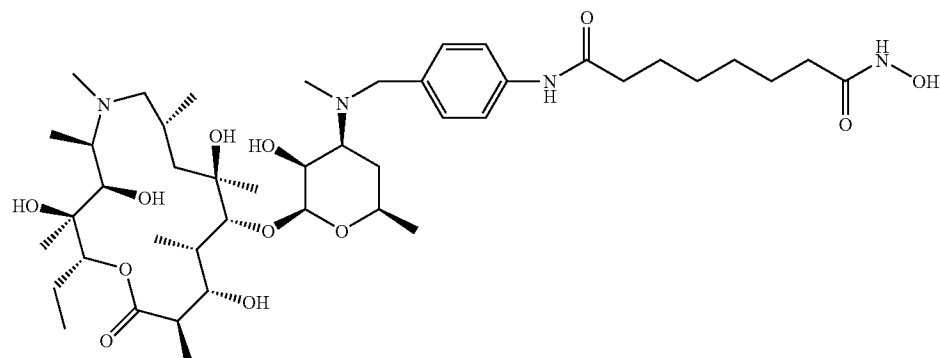
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
30	
36	
38	

TABLE 3-continued

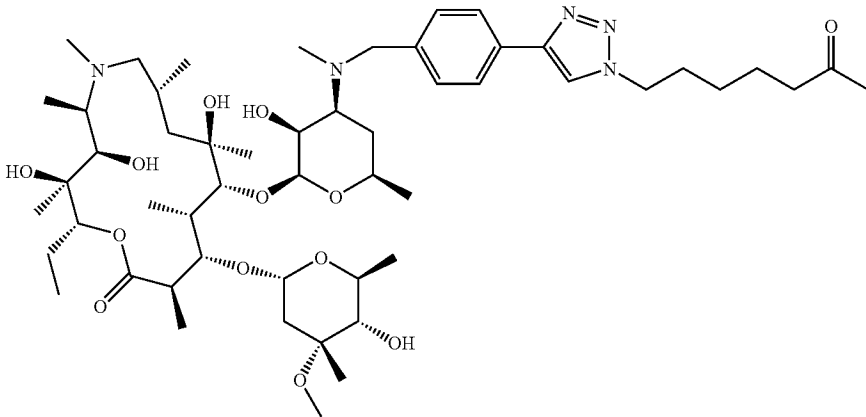
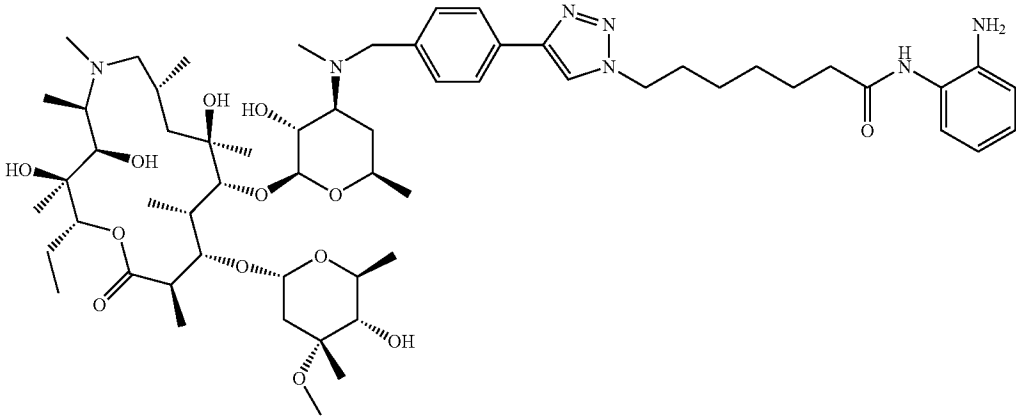
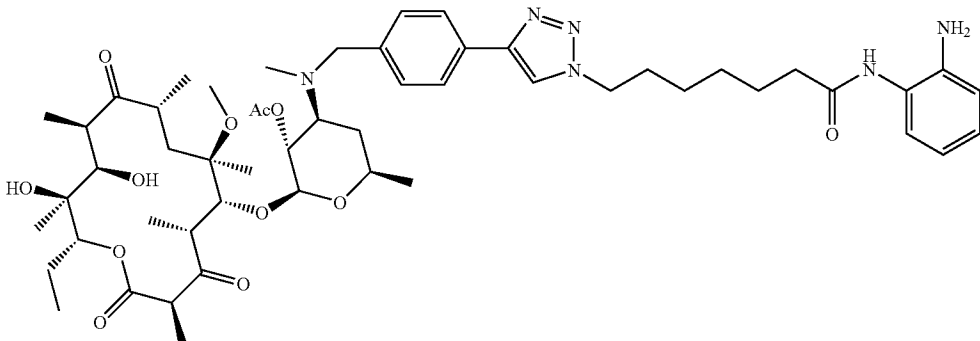
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
40	
44	
47	

TABLE 3-continued

COM- POUND NUMBER	Non-peptide HDAC Inhibitors
	STRUCTURE
48	
49	
50	

TABLE 3-continued

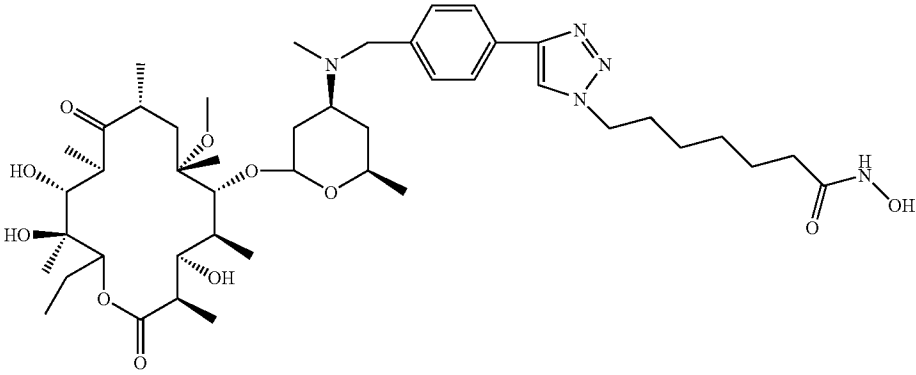
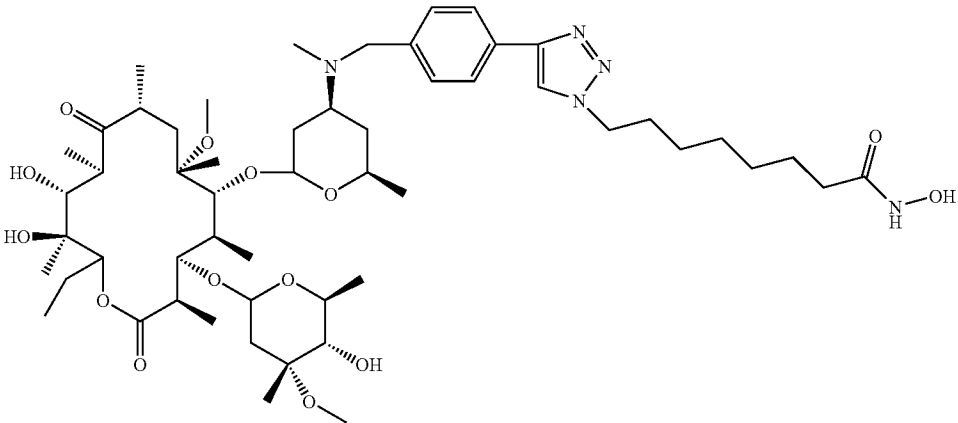
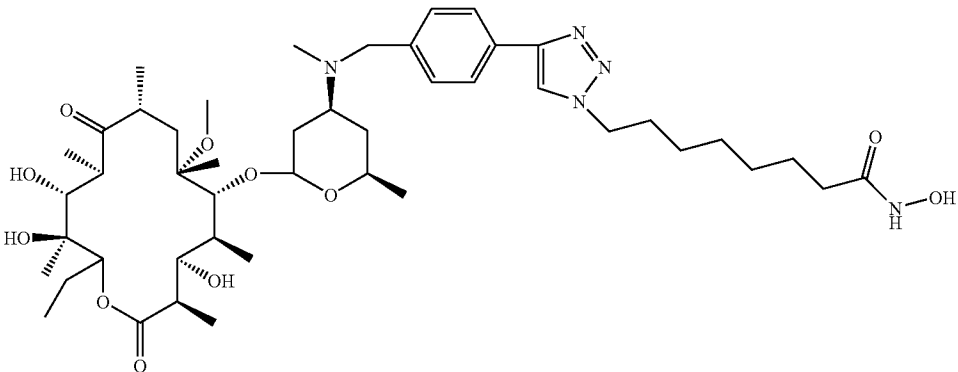
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
51	
52	
53	

TABLE 3-continued

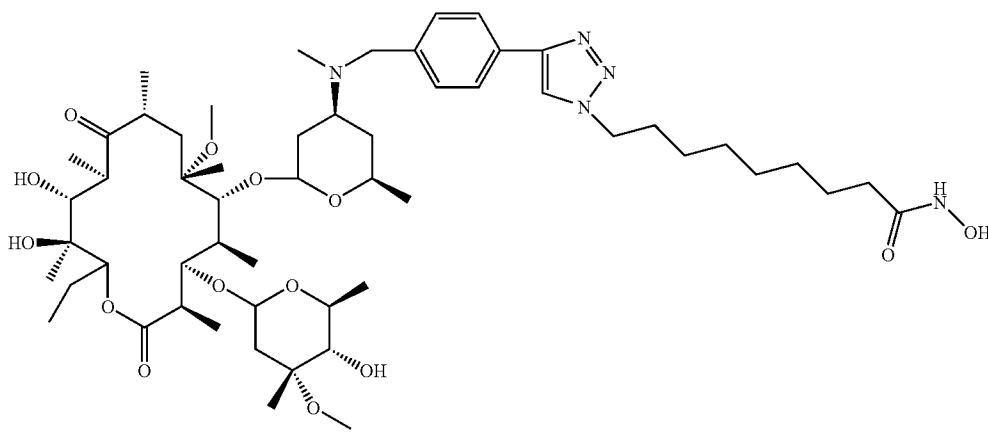
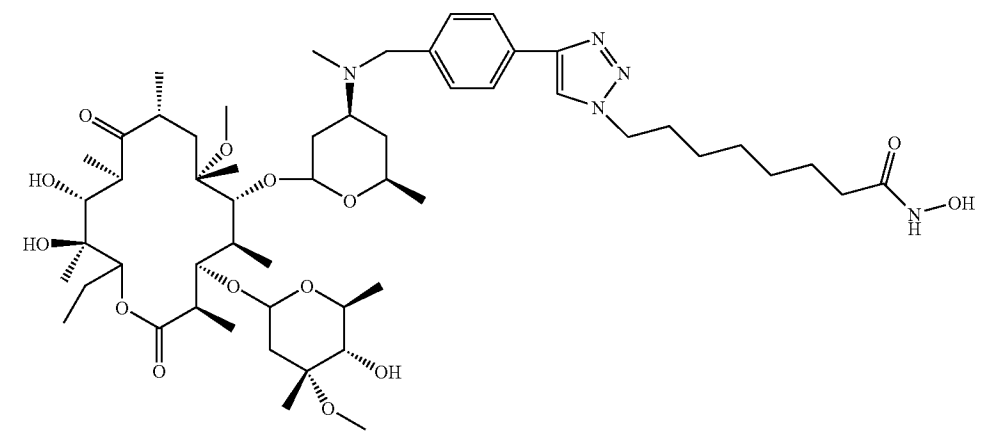
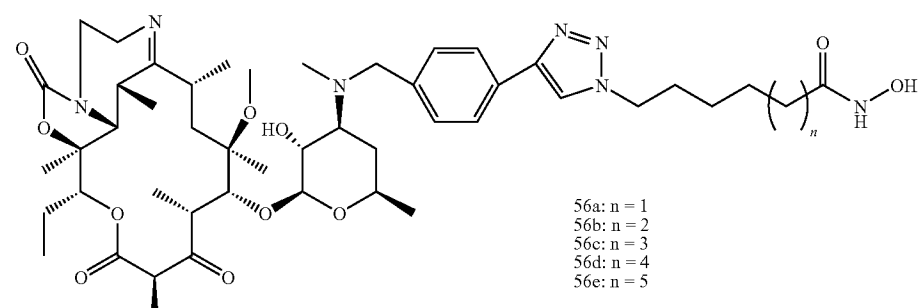
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
54	
55	
56	 <p>56a: n = 1 56b: n = 2 56c: n = 3 56d: n = 4 56e: n = 5</p>

TABLE 3-continued

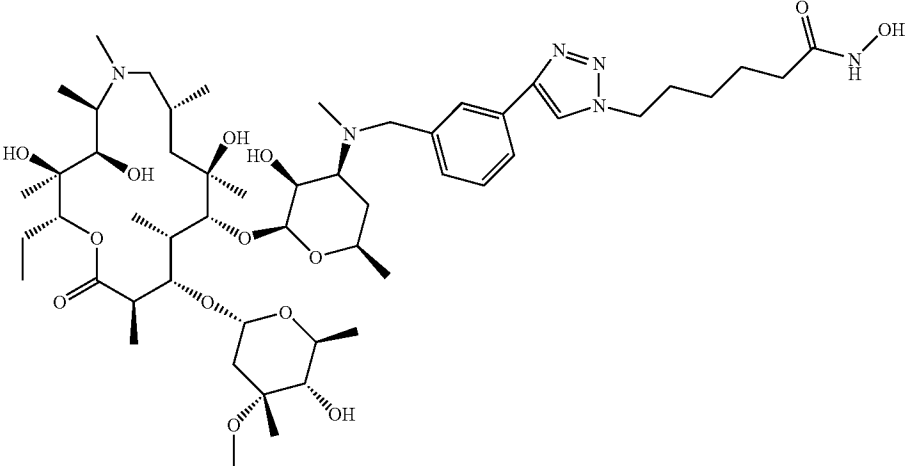
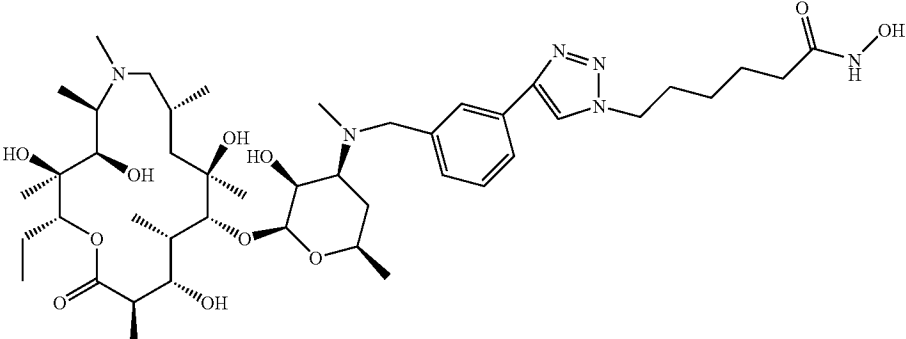
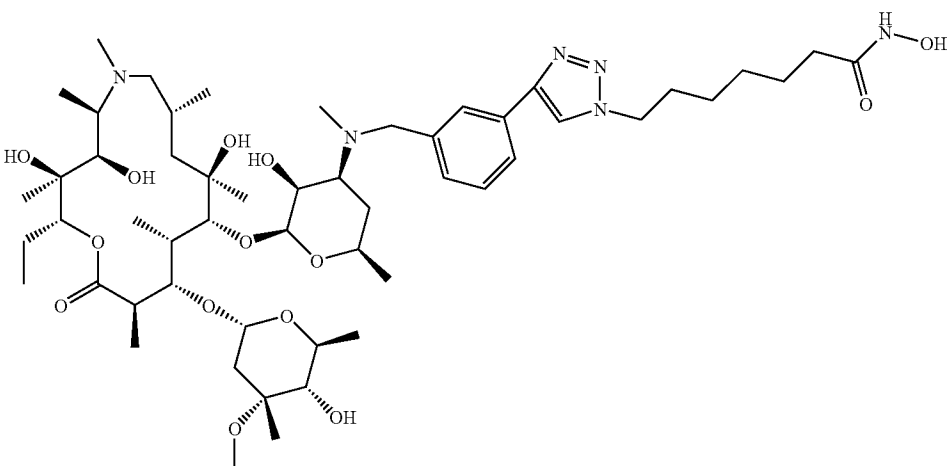
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
82	
83	
84	

TABLE 3-continued

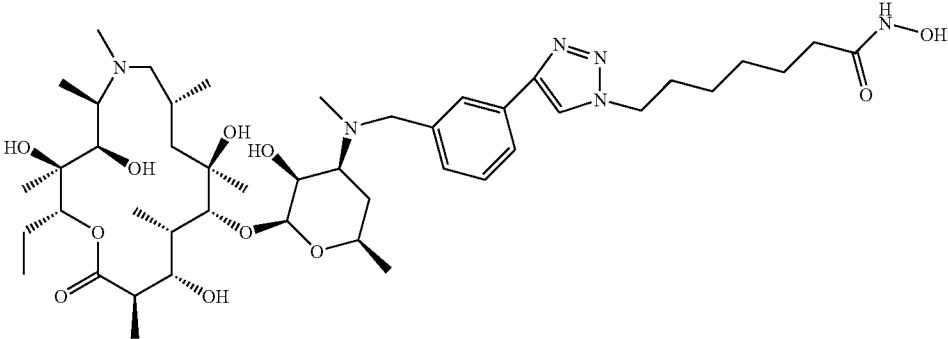
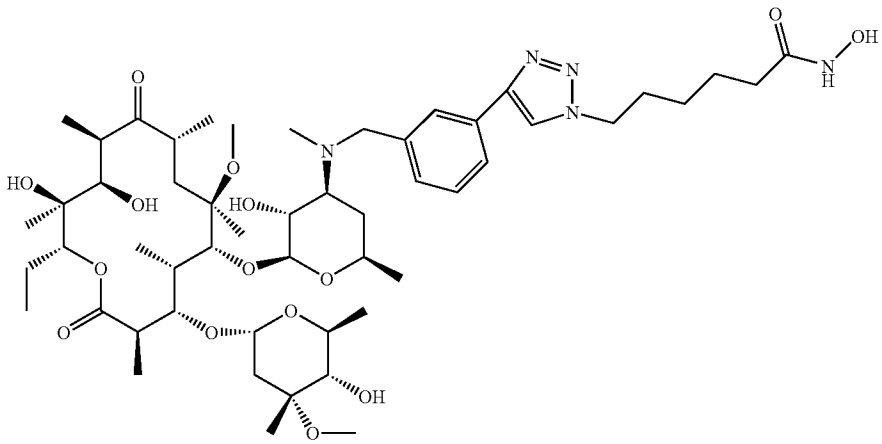
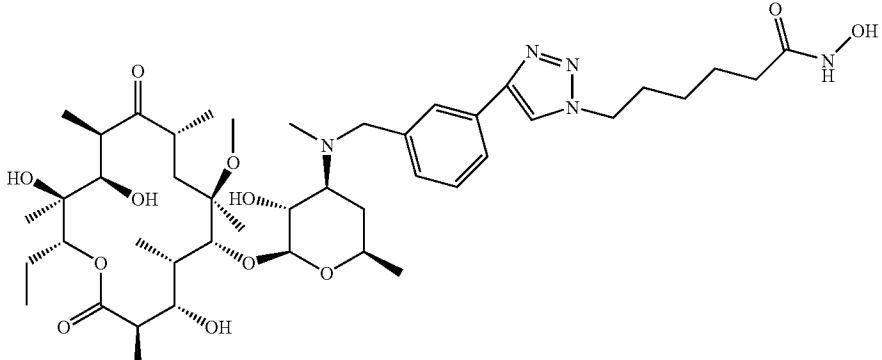
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
85	
86	
87	

TABLE 3-continued

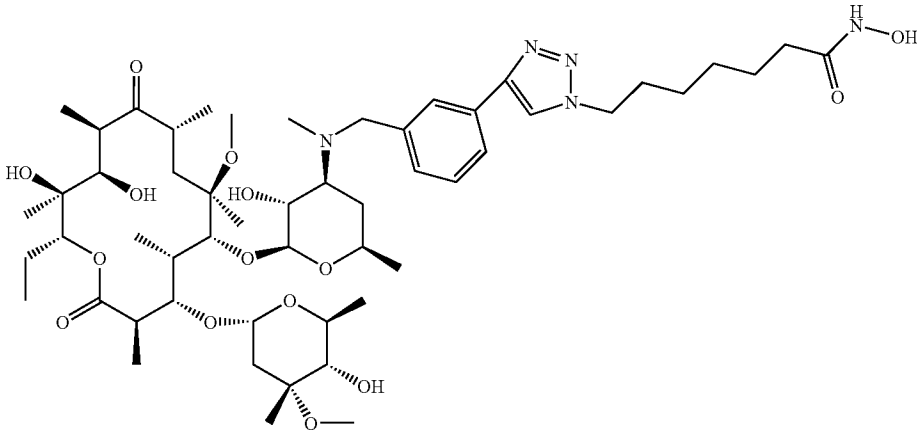
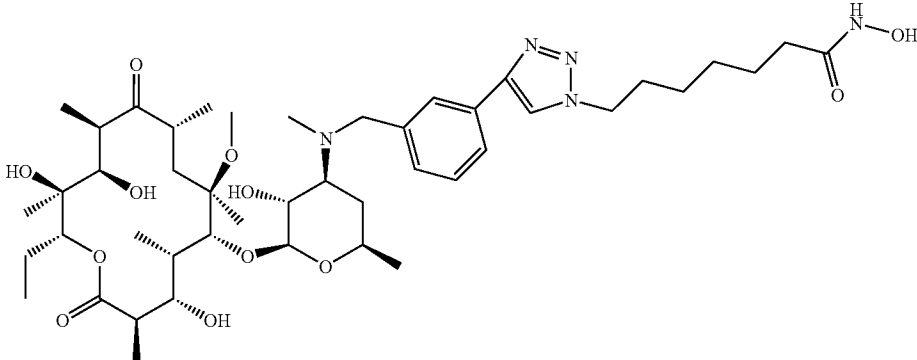
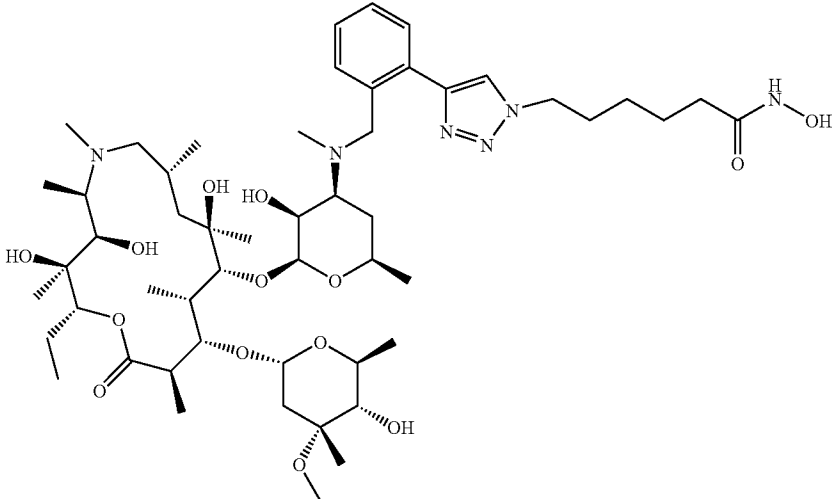
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
88	
89	
90	





TABLE 3-continued

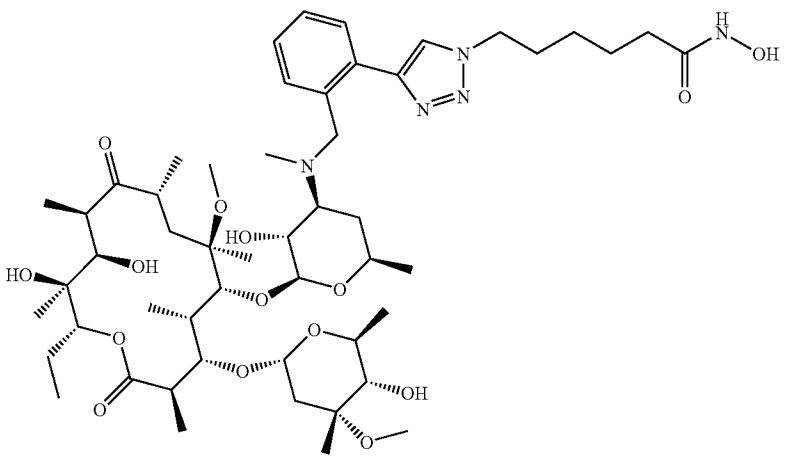
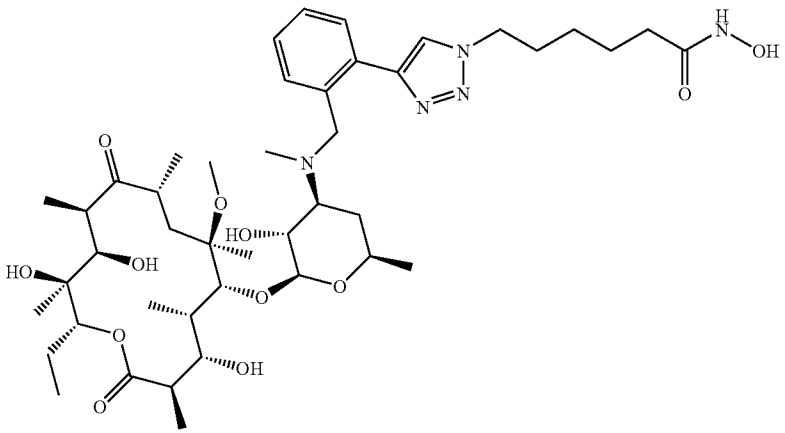
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
94	
95	
96	

TABLE 3-continued

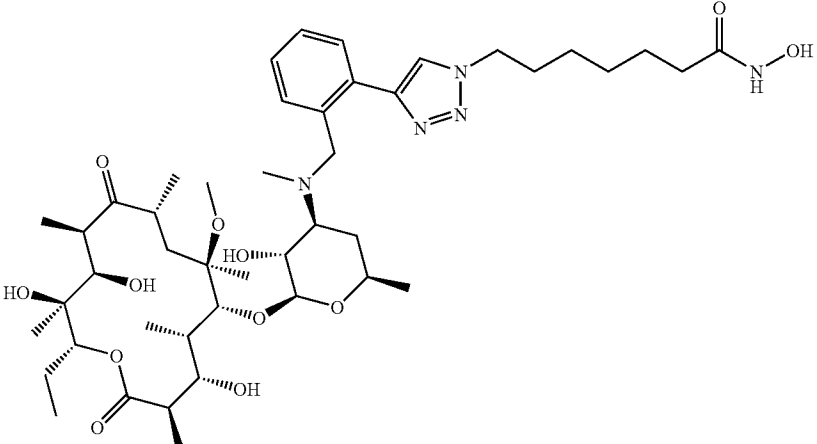
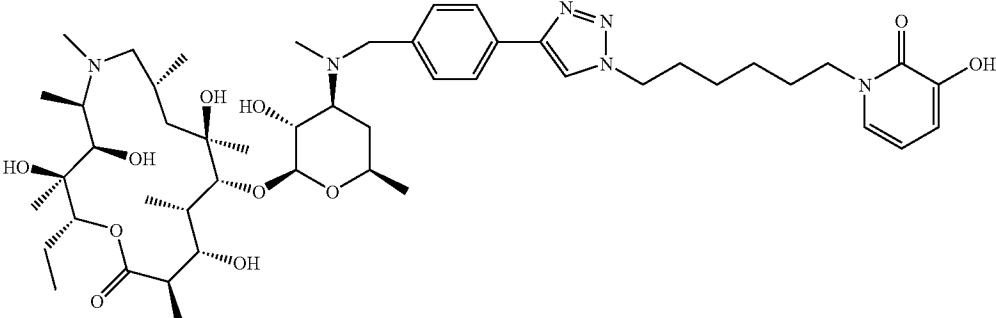
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
97	
98	
99	

TABLE 3-continued

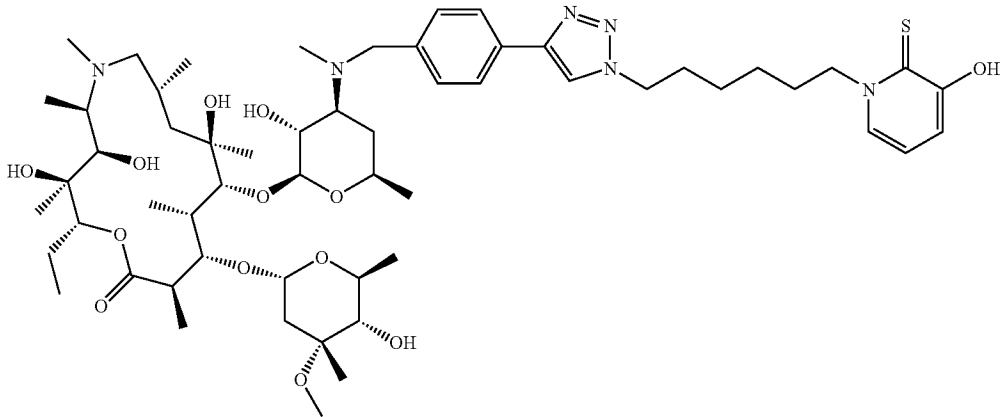
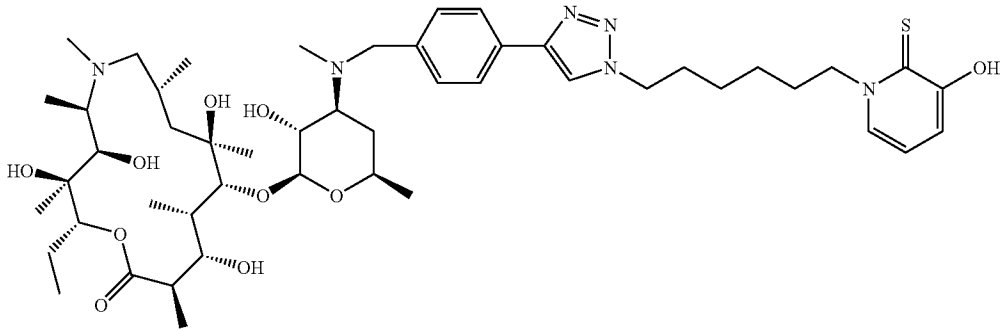
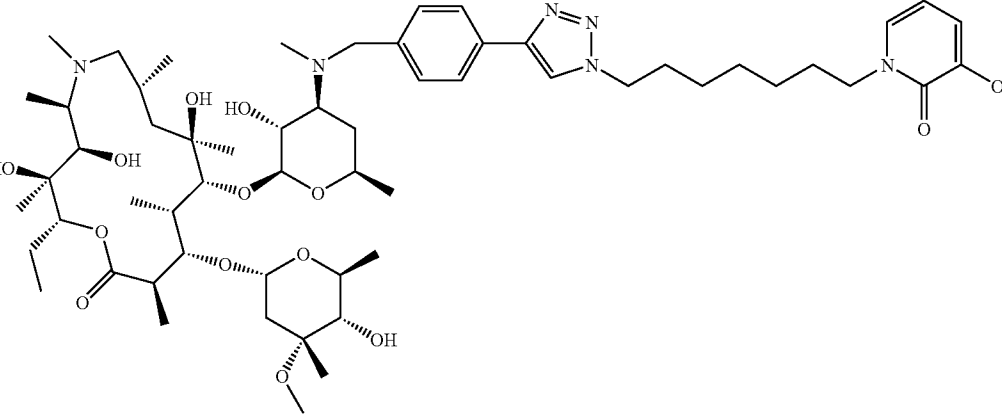
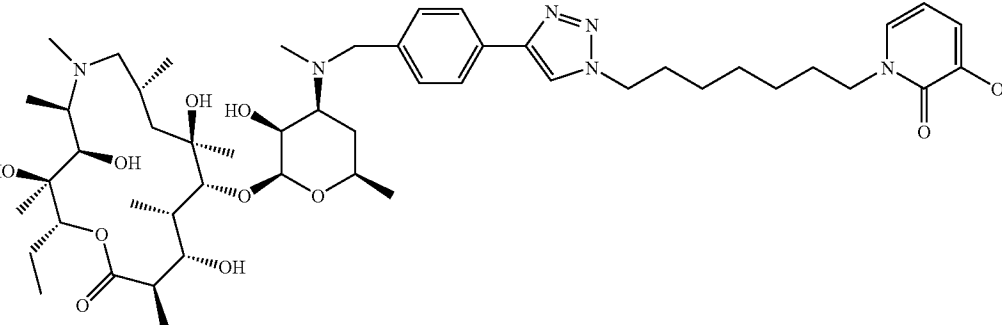
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
100	
101	
102	
103	

TABLE 3-continued

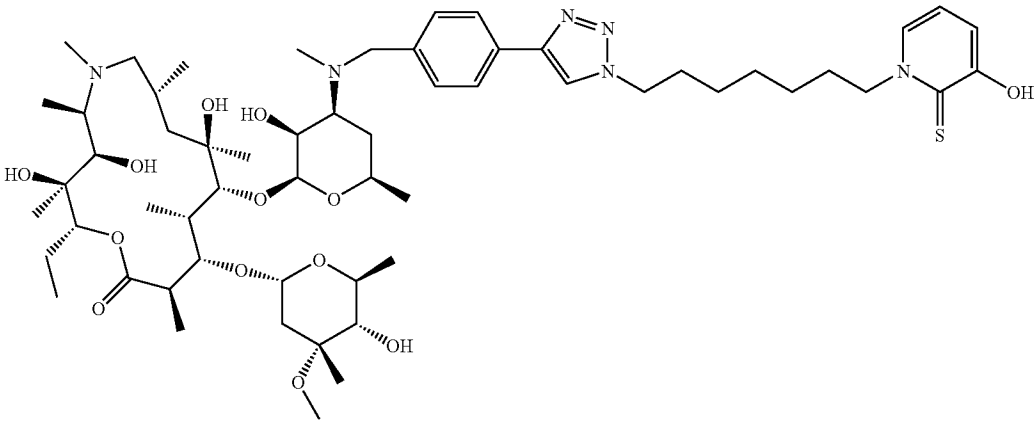
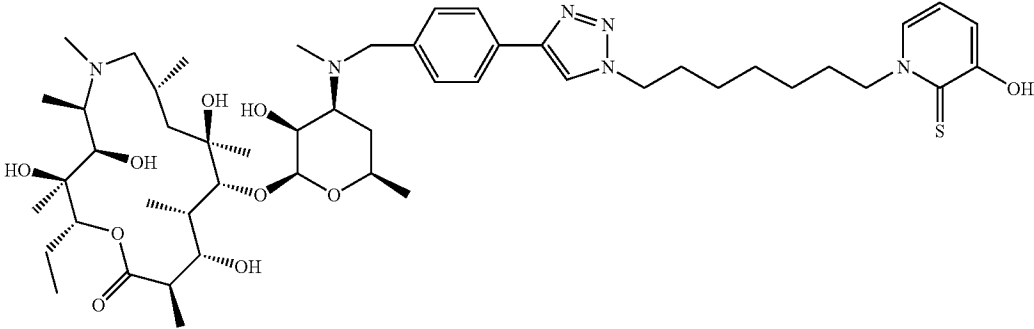
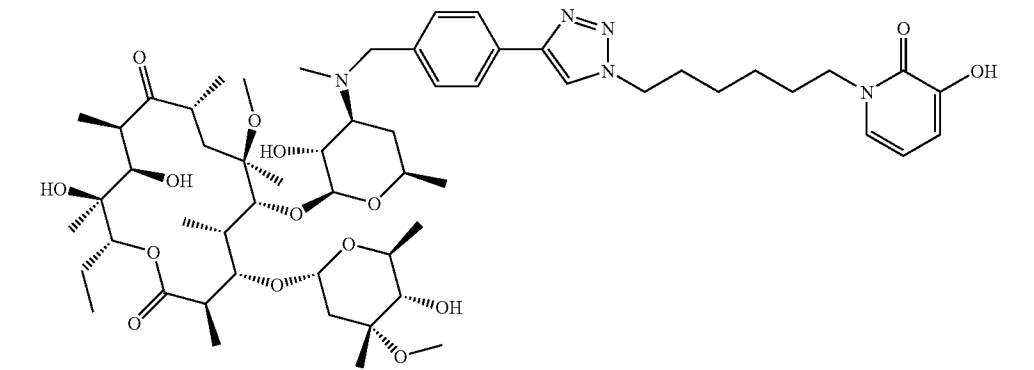
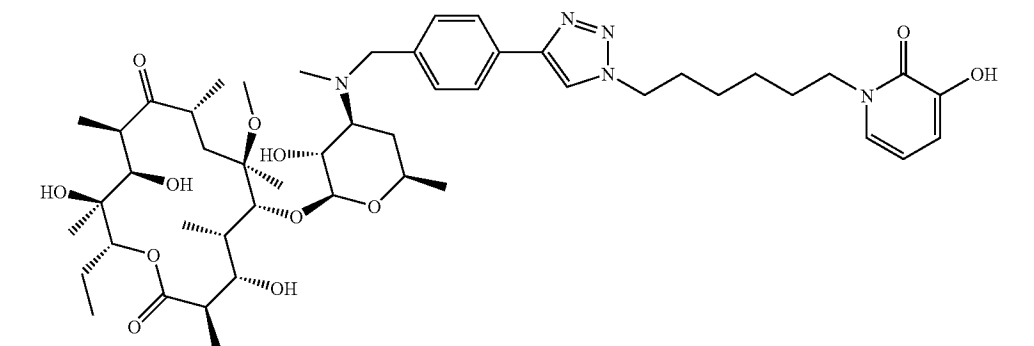
COM- POUND NUMBER	STRUCTURE
104	
105	
106	
107	

TABLE 3-continued

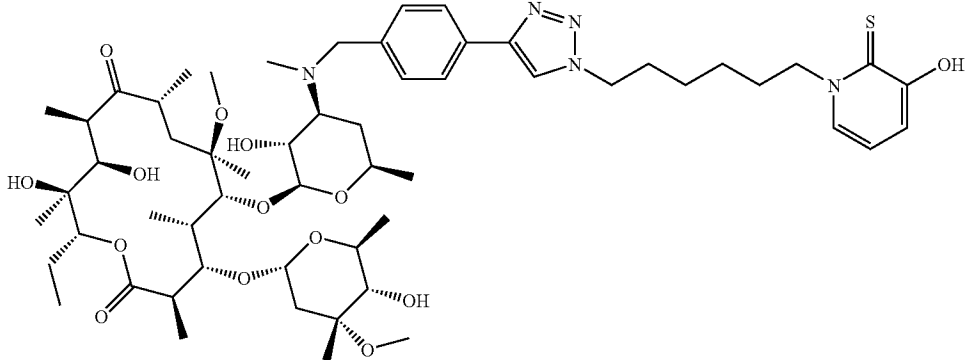
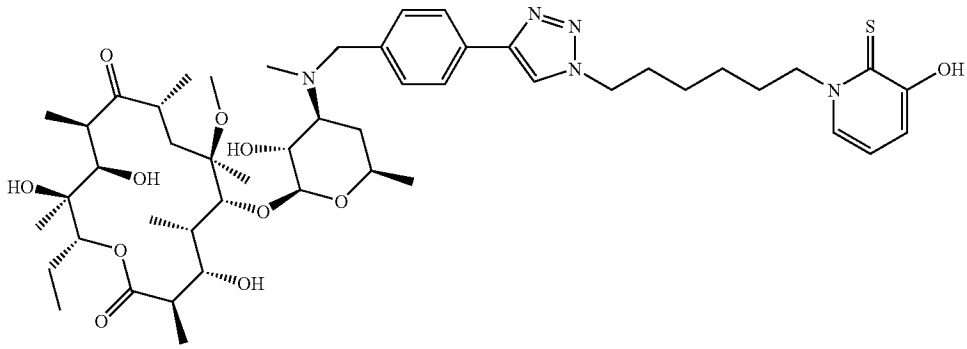
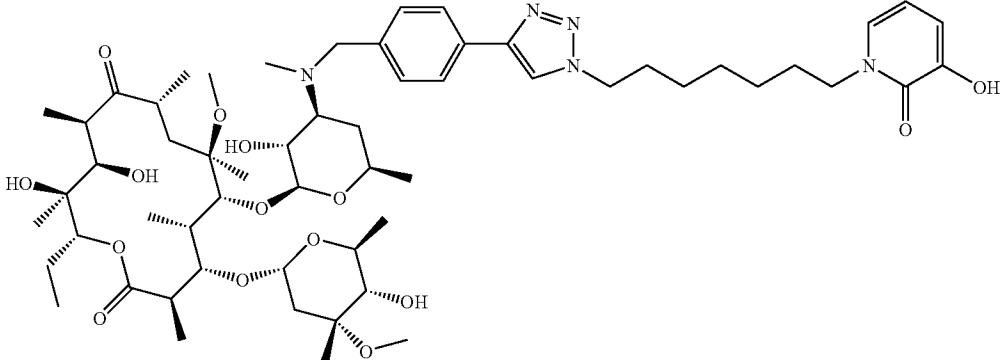
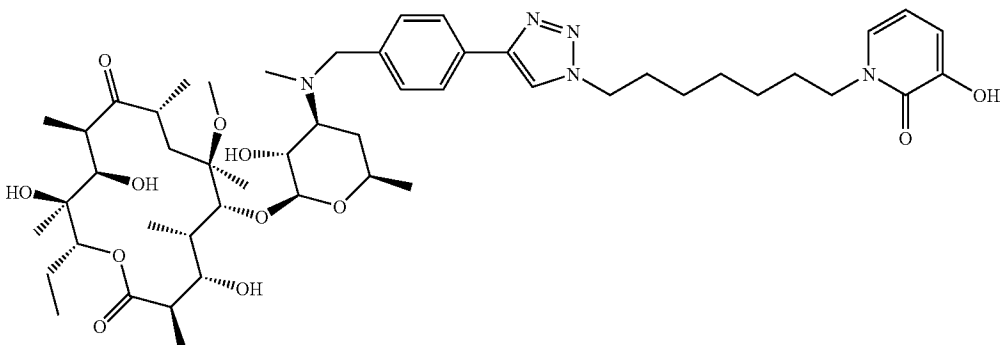
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
108	
109	
110	
111	

TABLE 3-continued

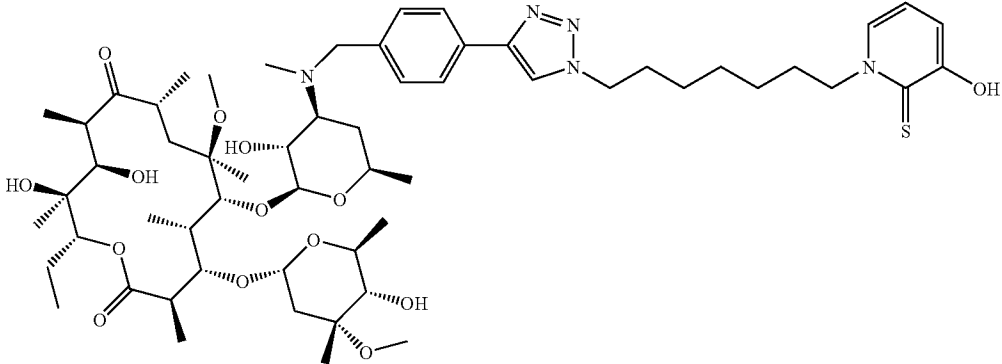
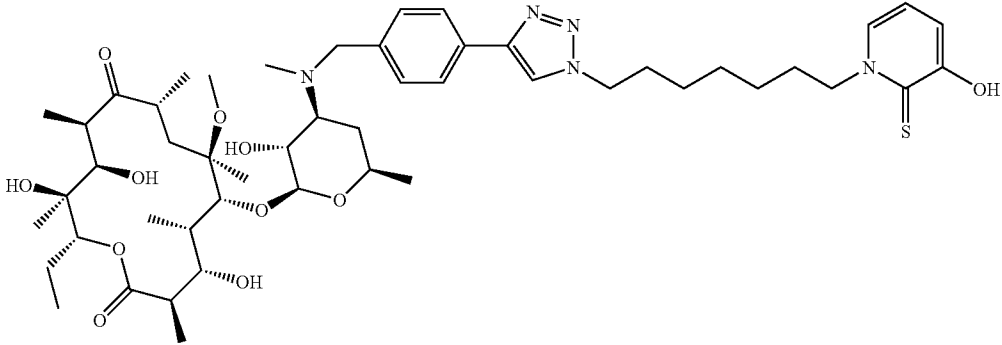
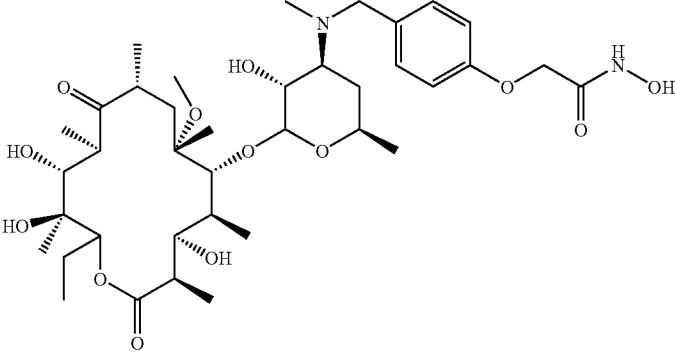
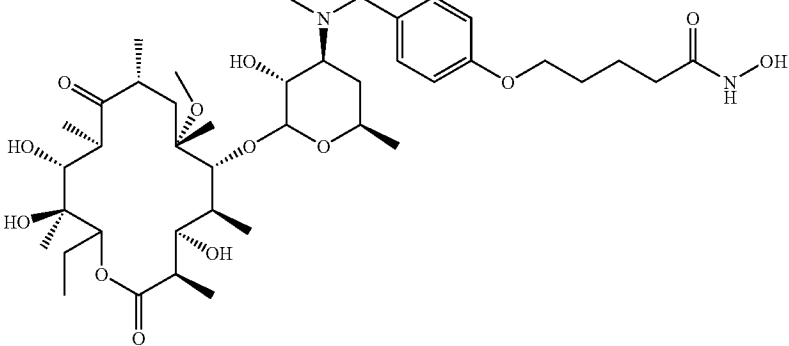
COM- POUND NUMBER	Non-peptide HDAC Inhibitors
	STRUCTURE
112	
113	
130	
131	

TABLE 3-continued

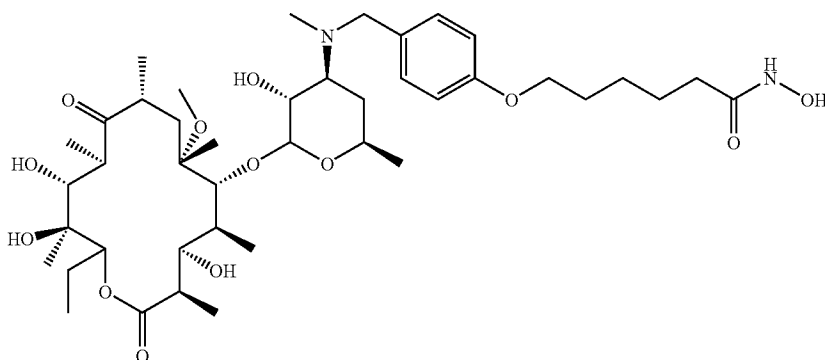
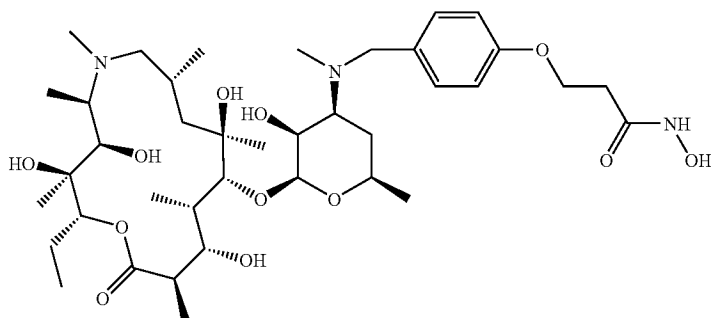
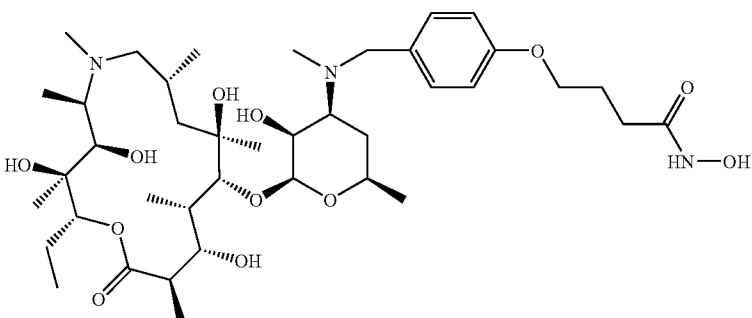
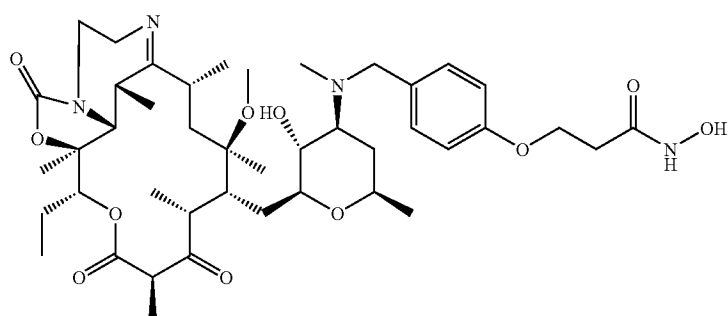
Non-peptide HDAC Inhibitors	
COM- POUND NUMBER	STRUCTURE
132	
133	
134	
136	



TABLE 3-continued

COM- POUND NUMBER	STRUCTURE
137	

It is believed that substitution of the cyclic peptide moiety of a prototypical cyclic-peptide HDAC inhibitor with macrolide skeletons will generate a new class of potent HDAC inhibitors. Furthermore, this class of HDAC inhibitors may possess targeted activity, such as targeted anti-cancer activity due to selective tissue distribution conferred by the macrolide moiety. The biological effects of macrolides are aided by their high distribution into target tissues. Macrolides accumulate in higher concentration within leukocytes as compared to levels found in serum.

### III. Formulations

The compounds described herein can be formulated for enteral, parenteral, and/or topical (e.g., transdermal, mucosal, etc.) administration. The compounds and their pharmaceutically-acceptable addition salts, prodrugs, and/or solvates can also be used in the form of pharmaceutical preparations which facilitate bioavailability. One or more compounds described herein may be administered in a single dosage form or in multiple dosage forms.

Formulations containing one or more of the compounds described herein may be prepared using a pharmaceutically acceptable carrier composed of materials that are considered safe and effective and may be administered to an individual without causing undesirable biological side effects or unwanted interactions. The carrier is all components present in the pharmaceutical formulation other than the active ingredient or ingredients. As generally used herein "carrier" includes, but is not limited to, diluents, binders, lubricants, disintegrants, fillers, pH modifying agents, preservatives, antioxidants, solubility enhancers, and coating compositions.

Carrier also includes all components of coating compositions which may include plasticizers, pigments, colorants, stabilizing agents, and glidants. Delayed release, extended release, and/or pulsatile release dosage formulations may be prepared as described in standard references such as "Pharmaceutical dosage form tablets", eds. Liberman et al. (New York, Marcel Dekker, Inc., 1989), "Remington—The science and practice of pharmacy", 20th ed., Lippincott Williams & Wilkins, Baltimore, Md., 2000, and "Pharmaceutical dosage forms and drug delivery systems", 6<sup>th</sup> Edition, Ansel et al., (Media, Pa.: Williams and Wilkins, 1995). These references provide information on carriers, materials, equipment and process for preparing tablets and capsules and delayed release dosage forms of tablets, capsules, and granules.

Examples of suitable coating materials include, but are not limited to, cellulose polymers such as cellulose acetate phthalate,

hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate and hydroxypropyl methylcellulose acetate succinate; polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins that are commercially available under the trade name EUDRAGIT® (Roth Pharma, Westerstadt, Germany), zein, shellac, and polysaccharides.

Additionally, the coating material may contain conventional carriers such as plasticizers, pigments, colorants, glidants, stabilization agents, pore formers and surfactants.

Optional pharmaceutically acceptable excipients present in the drug-containing tablets, beads, granules or particles include, but are not limited to, diluents, binders, lubricants, disintegrants, colorants, stabilizers, and surfactants. Diluents, also referred to as "fillers," are typically necessary to increase the bulk of a solid dosage form so that a practical size is provided for compression of tablets or formation of beads and granules. Suitable diluents include, but are not limited to, dicalcium phosphate dihydrate, calcium sulfate, lactose, sucrose, mannitol, sorbitol, cellulose, microcrystalline cellulose, kaolin, sodium chloride, dry starch, hydrolyzed starches, pregelatinized starch, silicone dioxide, titanium oxide, magnesium aluminum silicate and powdered sugar.

Binders are used to impart cohesive qualities to a solid dosage formulation, and thus ensure that a tablet or bead or granule remains intact after the formation of the dosage forms. Suitable binder materials include, but are not limited to, starch, pregelatinized starch, gelatin, sugars (including sucrose, glucose, dextrose, lactose and sorbitol), polyethylene glycol, waxes, natural and synthetic gums such as acacia, tragacanth, sodium alginate, cellulose, including hydroxypropylmethylcellulose, hydroxypropylcellulose, ethylcellulose, and veegum, and synthetic polymers such as acrylic acid and methacrylic acid copolymers, methacrylic acid copolymers, methyl methacrylate copolymers, aminoalkyl methacrylate copolymers, polyacrylic acid/polymethacrylic acid and polyvinylpyrrolidone.

Lubricants are used to facilitate tablet manufacture. Examples of suitable lubricants include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, glycerol behenate, polyethylene glycol, talc, and mineral oil.

Disintegrants are used to facilitate dosage form disintegration or "breakup" after administration, and generally include, but are not limited to, starch, sodium starch glycolate, sodium carboxymethyl starch, sodium carboxymethylcellulose, hydroxypropyl cellulose, pregelatinized starch, clays, cellu-

lose, alginine, gums or cross linked polymers, such as cross-linked PVP (Polypasdone XL from GAF Chemical Corp).

Stabilizers are used to inhibit or retard drug decomposition reactions which include, by way of example, oxidative reactions.

Surfactants may be anionic, cationic, amphoteric or non-ionic surface active agents. Suitable anionic surfactants include, but are not limited to, those containing carboxylate, sulfonate and sulfate ions. Examples of anionic surfactants include sodium, potassium, ammonium of long chain alkyl sulfonates and alkyl aryl sulfonates such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium dodecylbenzene sulfonate; dialkyl sodium sulfosuccinates, such as sodium bis-(2-ethylthioxy)-sulfosuccinate; and alkyl sulfates such as sodium lauryl sulfate. Cationic surfactants include, but are not limited to, quaternary ammonium compounds such as benzalkonium chloride, benzethonium chloride, cetrimonium bromide, stearyl dimethylbenzyl ammonium chloride, polyoxyethylene and coconut amine. Examples of nonionic surfactants include ethylene glycol monostearate, propylene glycol myristate, glyceryl monostearate, glyceryl stearate, polyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG-150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000 cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Poloxamer® 401, stearyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl- $\beta$ -alanine, sodium N-lauryl- $\beta$ -iminodipropionate, myristo-  
30 amphoacetate, lauryl betaine and lauryl sulfobetaine.

If desired, the tablets, beads, granules, or particles may also contain minor amount of nontoxic auxiliary substances such as wetting or emulsifying agents, dyes, pH buffering agents, or preservatives.

#### Enteral Formulations

Pharmaceutical compositions for oral administration can be liquid or solid. Liquid dosage forms suitable for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to an encapsulated or unencapsulated HDAC inhibitor, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents.

Solid dosage forms for oral administration include, but are not limited to, capsules, tablets, caplets, dragees, powders and granules. In such solid dosage forms, the encapsulated or unencapsulated compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid, (b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia, (c) humectants such as glycerol, (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate, (e) solution retarding agents such as par-

affin, (f) absorption accelerators such as quaternary ammonium compounds, (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, (h) absorbents such as kaolin and bentonite clay and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also contain buffering agents.

Solid compositions of a similar type may also be employed as fill materials in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art.

#### Parenteral Formulations

Pharmaceutical preparations in the form suitable for injection are subjected to conventional pharmaceutical operations such as sterilization and/or may contain adjuvants including, but not limited to, preservatives, stabilizers, wetting or emulsifying agents, and buffers.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated as known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension, or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils can be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectable formulations. In a particularly preferred embodiment, the compound is suspended in a carrier fluid containing 1% (w/v) sodium carboxymethyl cellulose and 0.1% (v/v) TWEEN™ 80. The injectable formulations can be sterilized, for example, by filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

#### Topical Formulations.

The compounds described here can also be formulated for topical, transdermal, or mucosal delivery. Dosage forms for topical or transdermal administration include, but are not limited to, ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, or patches. The compounds are typically admixed under sterile conditions with a pharmaceutically acceptable carrier and any excipients (e.g., preservatives, buffers, etc.) that may be required. Ophthalmic formulations, ear drops and eye drops can also be prepared. The ointments, pastes, creams and gels may contain, in addition to the active agent, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compounds described herein in a proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound(s) in a polymer matrix or gel.

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Powders and sprays can contain, in addition to the active agent, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these drugs. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds described herein with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol, or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the compound(s).

#### A. Other Active Agents

The HDAC inhibitors described herein can be administered adjunctively with other active compounds. These compounds included but are not limited to analgesics, anti-inflammatory drugs, antipyretics, antidepressants, antiepileptics, antihistamines, antimigraine drugs, antimuscarinics, anxiolytics, sedatives, hypnotics, antipsychotics, bronchodilators, anti-asthma drugs, cardiovascular drugs, corticosteroids, dopaminergics, electrolytes, gastro-intestinal drugs, muscle relaxants, nutritional agents, vitamins, parasymphathomimetics, stimulants, anorectics and anti-narcoleptics. "Adjunctive administration", as used herein, means the HDAC inhibitors can be administered in the same dosage form or in separate dosage forms with one or more other active agents.

Specific examples of compounds that can be adjunctively administered with the GDAC inhibitors include, but are not limited to, aceclofenac, acetaminophen, adomoxetine, almotriptan, alprazolam, amantadine, amcinonide, aminocyclopropane, amitriptyline, amolodipine, amoxapine, amphetamine, aripiprazole, aspirin, atomoxetine, azasetron, azatadine, beclomethasone, benactyzine, benoxaprofen, bempoprofen, betamethasone, bicipradine, bromocriptine, budesonide, buprenorphine, bupropion, buspirone, butorphanol, butriptyline, caffeine, carbamazepine, carbidopa, carisoprodol, celecoxib, chlordiazepoxide, chlorpromazine, choline salicylate, citalopram, clomipramine, clonazepam, clonidine, clonitazene, clorazepate, clotiazepam, cloxazolam, clozapine, codeine, corticosterone, cortisone, cyclobenzaprine, cyproheptadine, demexiptiline, desipramine, desomorphine, dexamethasone, dexanabinol, dextroamphetamine sulfate, dextromoramide, dextropropoxyphene, dezocine, diazepam, dibenzepin, diclofenac sodium, diflunisal, dihydrocodeine, dihydroergotamine, dihydromorphine, dimetacrine, divalproex, dizatriptan, dolasetron, donepezil, dothiepin, doxepin, duloxetine, ergotamine, escitalopram, estazolam, ethosuximide, etodolac, femoxetine, fenamates, fenoprofen, fentanyl, fludiazepam, fluoxetine, fluphenazine, flurazepam, flurbiprofen, flutazolam, fluvoxamine, frovatriptan, gabapentin, galantamine, gepirone, ginkgo biloba, granisetron, haloperidol, huperzine A, hydrocodone, hydrocortisone, hydromorphone, hydroxyzine, ibuprofen, imipramine, indiplon, indomethacin, indoprofen, iprindole, ipsapirone, ketaserin, ketoprofen, ketorolac, lesopitron, levodopa, lipase, lofepramine, lorazepam, loxapine, maprotiline, mazindol, mefenamic acid, melatonin, melitracen, memantine, meperidine, meprobamate, mesalamine, metapramine, metaxalone, methadone, methadone, methamphetamine, methocarbamol, methylidopa, methylphenidate, methylsalicylate, methysergid(e), metoclopramide, mianserin, mifepristone, milnacipran, minaprine, mirtazapine, moclobemide, modafinil (an anti-narcoleptic), molindone, morphine, morphine hydrochloride, nabumetone, nadolol, naproxen, naratriptan, nefazodone, neurontin, nomifensine, nortriptyline, olanzapine, olsalazine, ondansetron, opipramol, orphenadrine, oxaflozane, oxaprazin, oxazepam, oxitriptan, oxycodone, oxymor-

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phone, pancrelipase, parecoxib, paroxetine, pemoline, pentazocine, pepsin, perphenazine, phenacetin, phendimetrazine, phenmetrazine, phenylbutazone, phenylloin, phosphatidylserine, pimozone, pirlindole, piroxicam, pizotifen, pizotyline, pramipexole, prednisolone, prednisone, pregabalin, propamolol, propizipine, propoxyphene, protriptyline, quazepam, quinupramine, reboxetine, reserpine, risperidone, ritanserine, rivastigmine, rizatriptan, rofecoxib, ropinirole, rotigotine, salsalate, sertraline, sibutramine, sildenafil, sulfasalazine, sulindac, sumatriptan, tacrine, temazepam, tetrabenazine, thiazides, thioridazine, thiothixene, tiapride, tiassipirone, tizanidine, tofenacin, tolmetin, toloxatone, topiramate, tramadol, trazodone, triazolam, trifluoperazine, trimethobenzamide, trimipramine, tropisetron, valdecoxib, valproic acid, venlafaxine, viloxazine, vitamin E, zimeldine, ziprasidone, zolmitriptan, zolpidem, zopiclone and isomers, salts, and combinations thereof.

#### IV. Methods of Preparation

Compound numberings, e.g., 1, 2, 3, etc. as used below are for reference within this section only are not to be confused with any similar numberings in the synthetic schemes described below or in the examples.

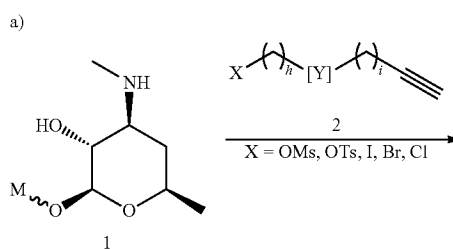
The schemes below describe exemplary chemistries that can be used to synthesize the compounds described herein. It is to be appreciated the compounds described herein may be synthesized by other methods known in the art.

#### General Synthetic Schemes

Scheme 1a-c illustrates representative general syntheses of compounds of types 5 and 6, 9 and 10, and 12 and 13. The starting des-N-methyl macrolide 1 could be sourced from a variety of N-demethylation reactions of the tertiary amines of basic sugars on macrolides known in the art (see Flynn et al. (1954) *J. Am. Chem. Soc.*, 76: 3121; U.S. Pat. No. 3,725,385; Ku et al., (1997) *Bioorg. Med. Chem. Lett.*, 7: 1203; Stenmark et al. (2000) *J. Org. Chem.*, 65: 3875; Randolph et al. (2004) *J. Med. Chem.*, 47, 1085; and U.S. Pat. No. 7,335,753).

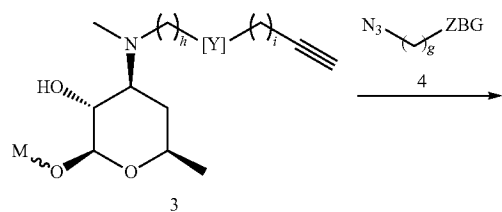
Reaction of 1 with electrophiles 2 and 7 yields alkynes and nitriles 3 and 8 respectively. Reactions of azide 4 and nitrile oxide 11 with alkynes 3 generate two regioisomeric triazole and isoxazole products 5 and 6 and 12 and 13 respectively. The triazole products' regioisomeric ratios and reaction rates could be altered by heating the reaction and/or by the use of catalysts (such as, but not limited to, copper (I) and Ru (II) salts and complexes: see Rostovtsev et al. (2002) *Angew. Chem. Int. Ed.*, 41: 2596; Tomoe et al. (2002) *J. Org. Chem.*, 67: 3057; Zhang et al. (2005) *J. Am. Chem. Soc.*, 127: 15998). Similarly, reaction of nitrile 8 and azide 4 generates two regioisomeric tetrazole products, 9 and 10. The ZBG in azide 4 can be protected if necessary. Suitable ZBG protecting groups include are described herein.

Scheme 1

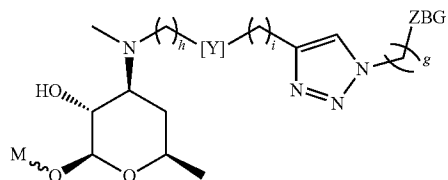


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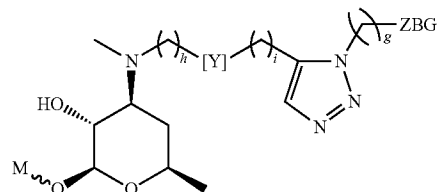
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3

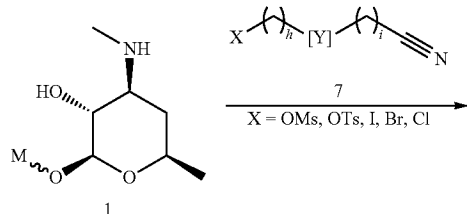


5 (1,4-isomer)

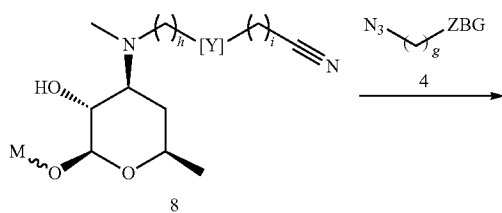


6 (1,5-isomer)

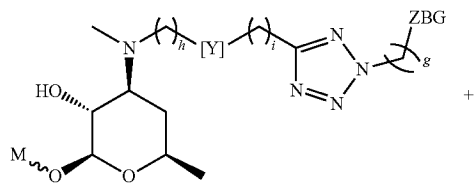
b)



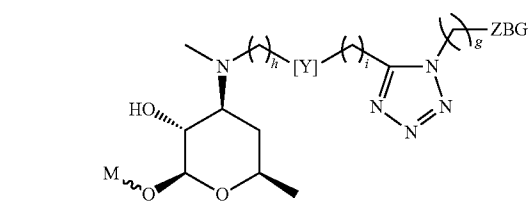
1



8



9 (1,4-isomer)

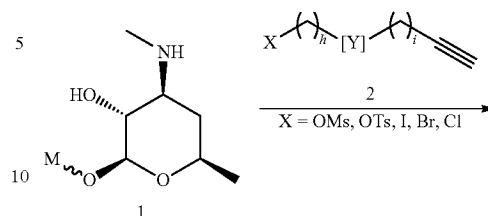


10 (1,5-isomer)

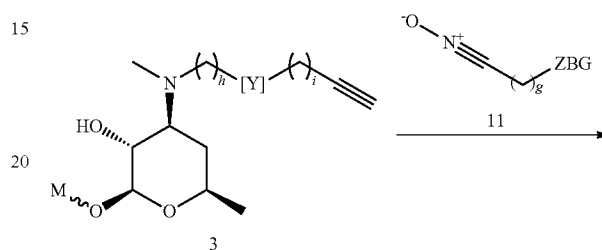
## 70

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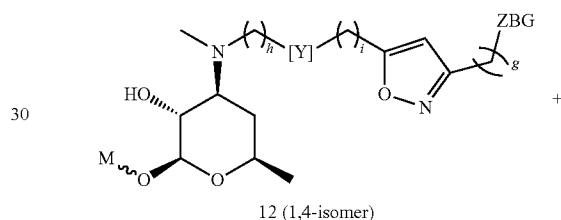
c)



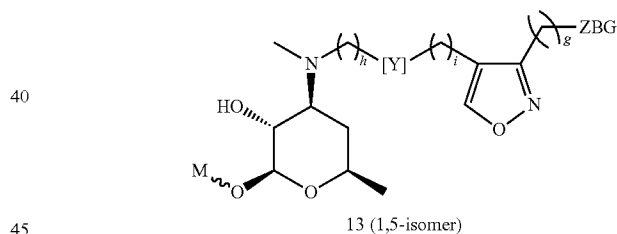
1



3



12 (1,4-isomer)



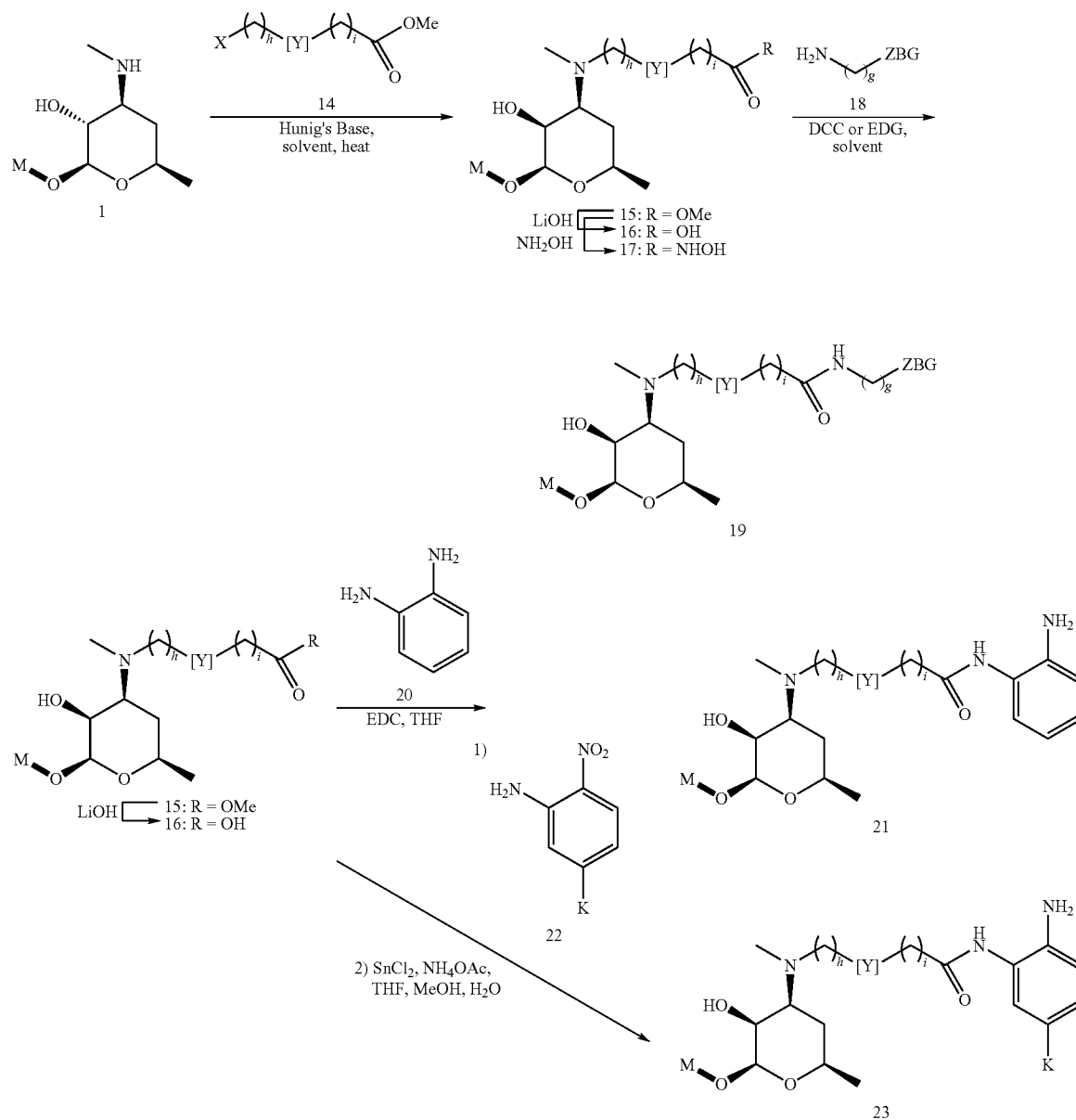
13 (1,5-isomer)

Scheme 2 illustrates representative general syntheses of compounds with amide as the linker-cap group connection moiety and benzamide compounds of types 17 and 19 and 21 and 23 respectively. Reaction of 1 with electrophile 14 yields ester 15. Compound 15 can be directly reacted with ZBG amine 18 to yield compound 19. Alternatively, 19 can be obtained from acid 16 and ZBG amine 18 through carbodiimide coupling. Additionally, hydroxamate 17, containing an appropriate aromatic moiety and appropriate alkyl chain length can be obtained from the reaction of hydroxylamine with ester 15. Similarly, benzamide compounds 21 and 23 can be prepared from the reaction of ester 15 or acid 16 with anilines 20 and 22. In the case of the para-substituted benzamide 23, the intermediate nitro anilide must be reduced to obtain the desired benzamide 23. The synthesis of para-substituted nitro aniline 22 is known in the art (for example, see Moradei et al. (2007) *J. Med. Chem.*, 45, 5543).

71

72

Scheme 2

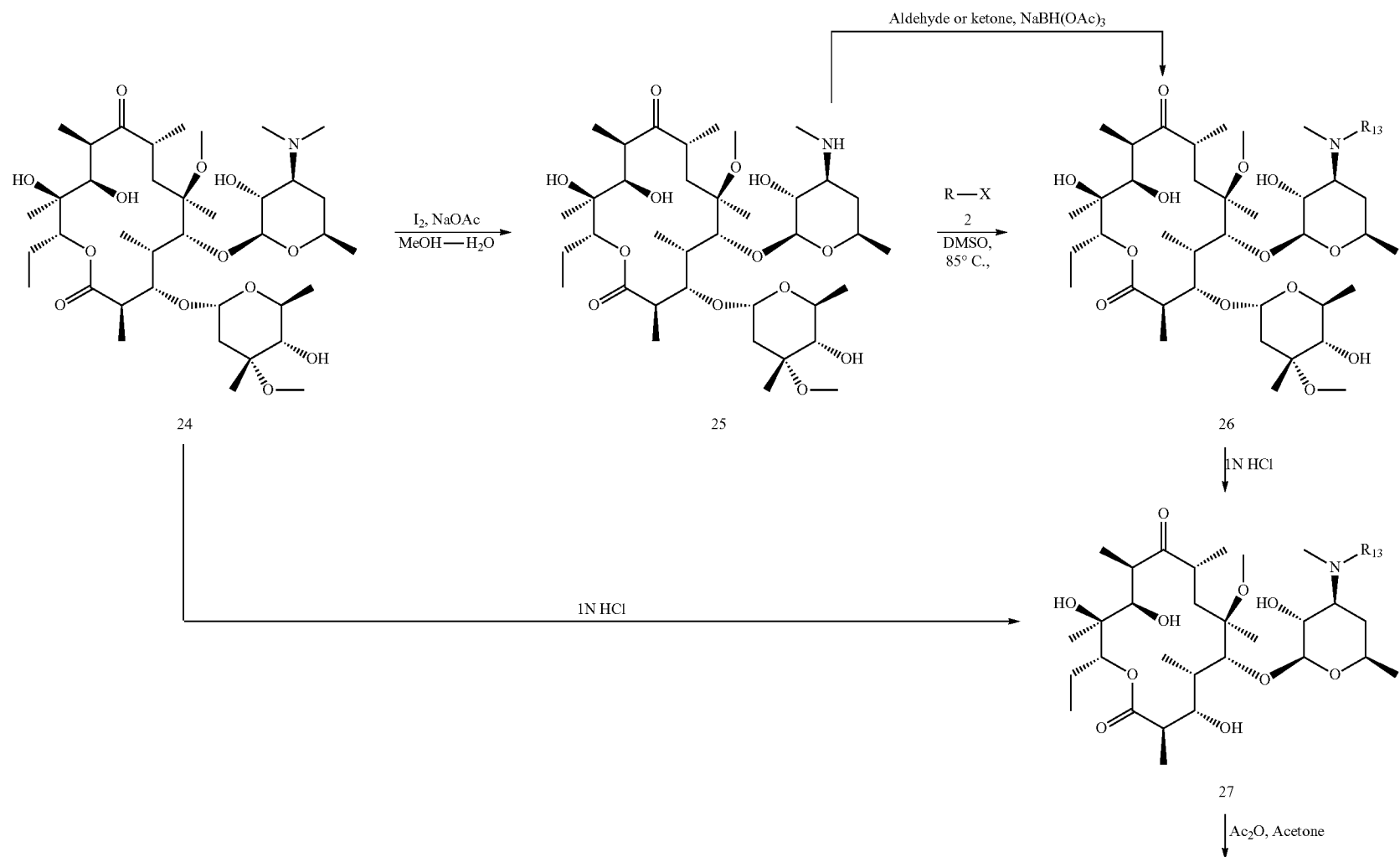


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Scheme 3 illustrates representative general syntheses of ketolide and bridged-ketolide based triazole derivatives. Clarithromycin 24 can be N-demethylated to give des-N-methyl clarithromycin 25. Reaction of 25 with electrophile 2 yields alkyne 26. Alternatively, alkyne 26 can be obtained by reductive amination of appropriate aldehydes and ketones. Descladinose 27 can be prepared from the treatment of alkyne 26 with dilute mineral acid, such as HCl. Compound 27, where  $\text{R}_{13}$  is  $\text{CH}_3$ , can be similarly obtained from reaction of 24 with dilute mineral acid. Selective acylation of the hydroxyl group of the amine sugar can be achieved by treatment of compound 27 with acetic anhydride in appropriate non-protic solvents such as, but not limited to, acetone, in the absence of base to yield compound 28. Oxidation of 28 under Corey-Kim or similar conditions (see Corey & Kim (1972) *J. Am. Chem. Soc.*, 94: 7586; Pfitzner & Moffatt (1965) *J. Am. Chem. Soc.*, 87: 5661; Ley et al. (1994) *Synthesis*, 639) leads

to ketolide 29. Treatment of 29 with carbonyldiimidazole and NaHMDS will give carbamate 30. Reaction of 30 with amines 31, 32 and ethane-1,2-diamine will afford intermediate 33, 34, and 35, respectively (see U.S. Pat. No. 5,631,355; Kashimura et al. (2003), *J. Antibiot.*, 56: 1062; Randolph et al. (2004) *J. Med. Chem.*, 47, 1085; Plata et al. (2004) *Tetr.*, 60: 10171). Subsequent reactions of intermediates 33, 34, and 35 with azide 4 will lead to bridged-ketolides 36 and 37, 38 and 39, and 40 and 41, respectively. When  $\text{R}_{13}$  contains an appropriate aromatic moiety and alkyl chain length, ketolide 29 can be modified to form compound 30 by methanolysis at elevated temperatures. Subsequent reaction of 30 with azide 4 will furnish ketolide 43. Again, it should be appreciated that the ZBG in azides of type 4 can be appropriately protected. Moreover, similar chemistries can be applied for the synthesis of the tetrazoles and oxazoles analogs of the ketolide and bridged-ketolide exemplified in scheme 3.

Scheme 3

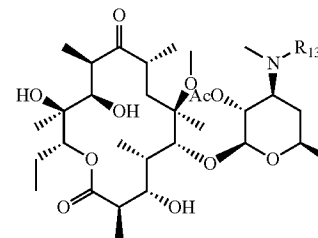


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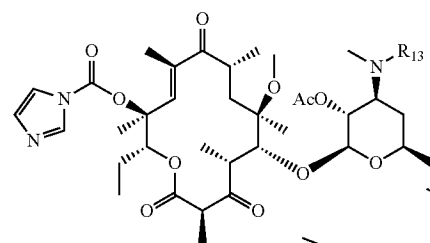
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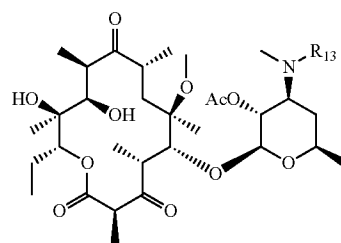
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28  
NCS, methyl sulfide,  
Et<sub>3</sub>N, or  
EDC, HCl, DMSO,  
pyridinium trifluoroacetate



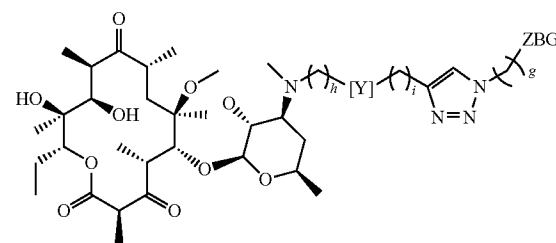
31

CDI,  
NaHMDS



MeOH,  
heat  
29: R = Ac  
42: R = H

N<sub>3</sub>-ZBG  
4



43

Z-NH<sub>2</sub>  
CH<sub>3</sub>CN/H<sub>2</sub>O,  
heat

H<sub>2</sub>N-[Y]-[Y]-[Y]  
30  
CH<sub>3</sub>CN/H<sub>2</sub>O,  
heat  
32

1. H<sub>2</sub>N-[Y]-[Y]-[Y]  
CH<sub>3</sub>CN/H<sub>2</sub>O,  
heat  
2. MeOH, sealed-tube, heat  
3. EtOH, AcOH, sealed-tube,  
heat

75

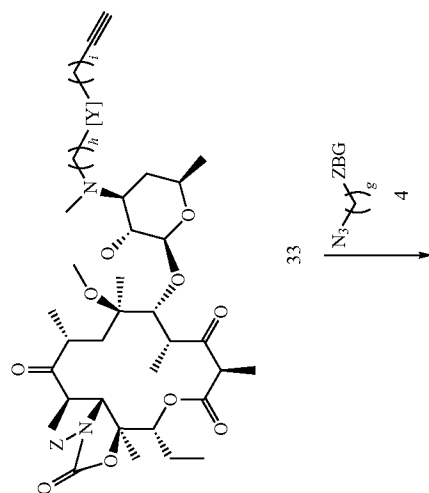
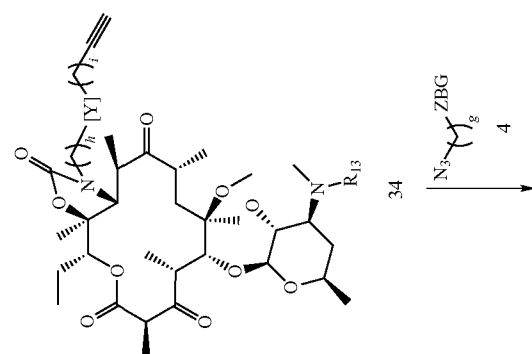
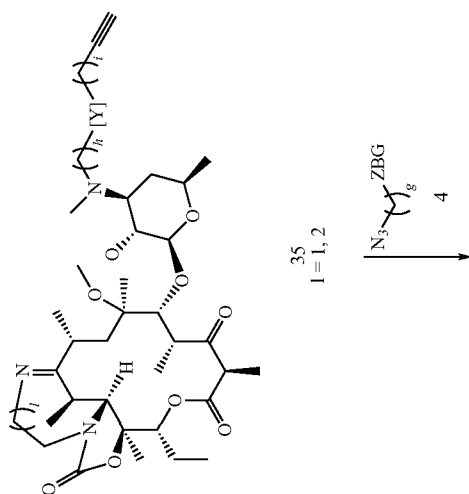
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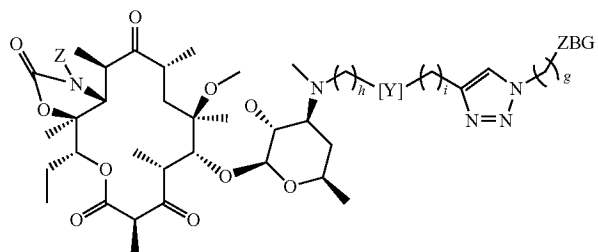
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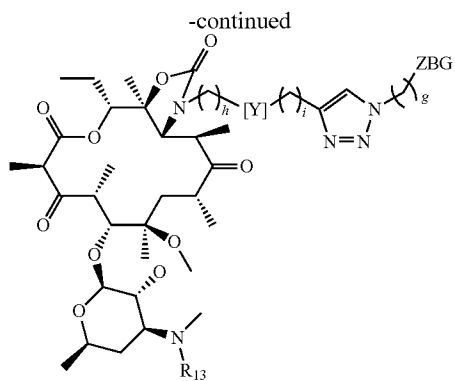
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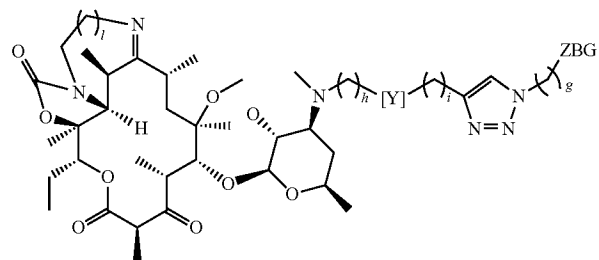




36  
+  
37 (1,5-isomer)



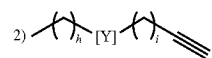
39  
+  
38 (1,5-isomer)



40  
+  
41 (1,5-isomer)

R<sub>13</sub> is

1) C1-C6 alkyl, optionally attached to nitrogen through primary or secondary carbon



[Y] is —CH<sub>2</sub>—, heteroatom aryl, Ar

h = 1-6

h = 2-6, when [Y] is heteroatom

i = 0-6

g = 1-10

Z is

1) H

2) C1-C6 alkyl, alkenyl, or alkynyl, optionally substituted with aryl, biphenyl or fused aryl groups

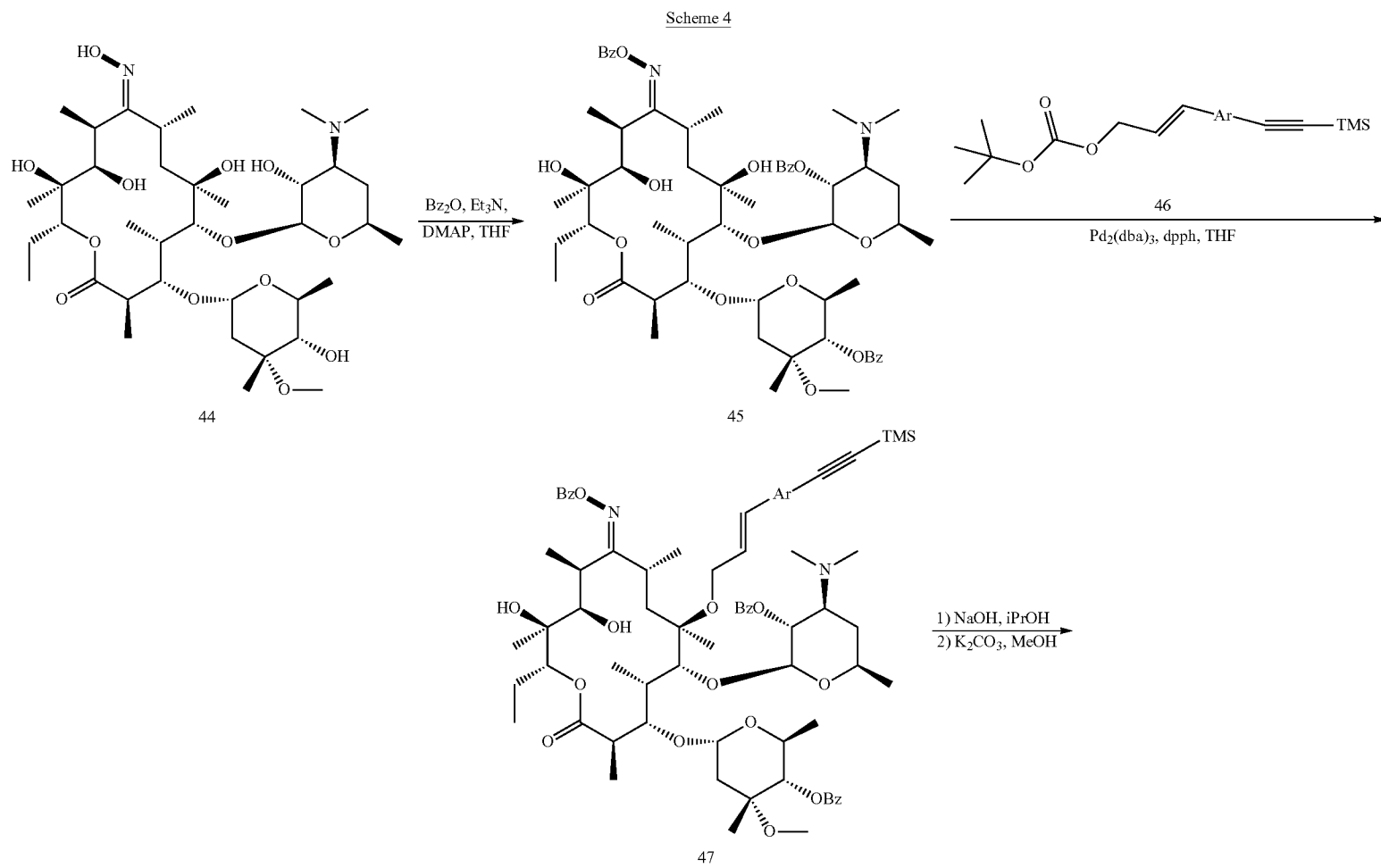
3) NHR, where R is H, C1-C6 alkyl, alkenyl, or alkynyl, optionally substituted with aryl, biphenyl or fused aryl groups

**81**

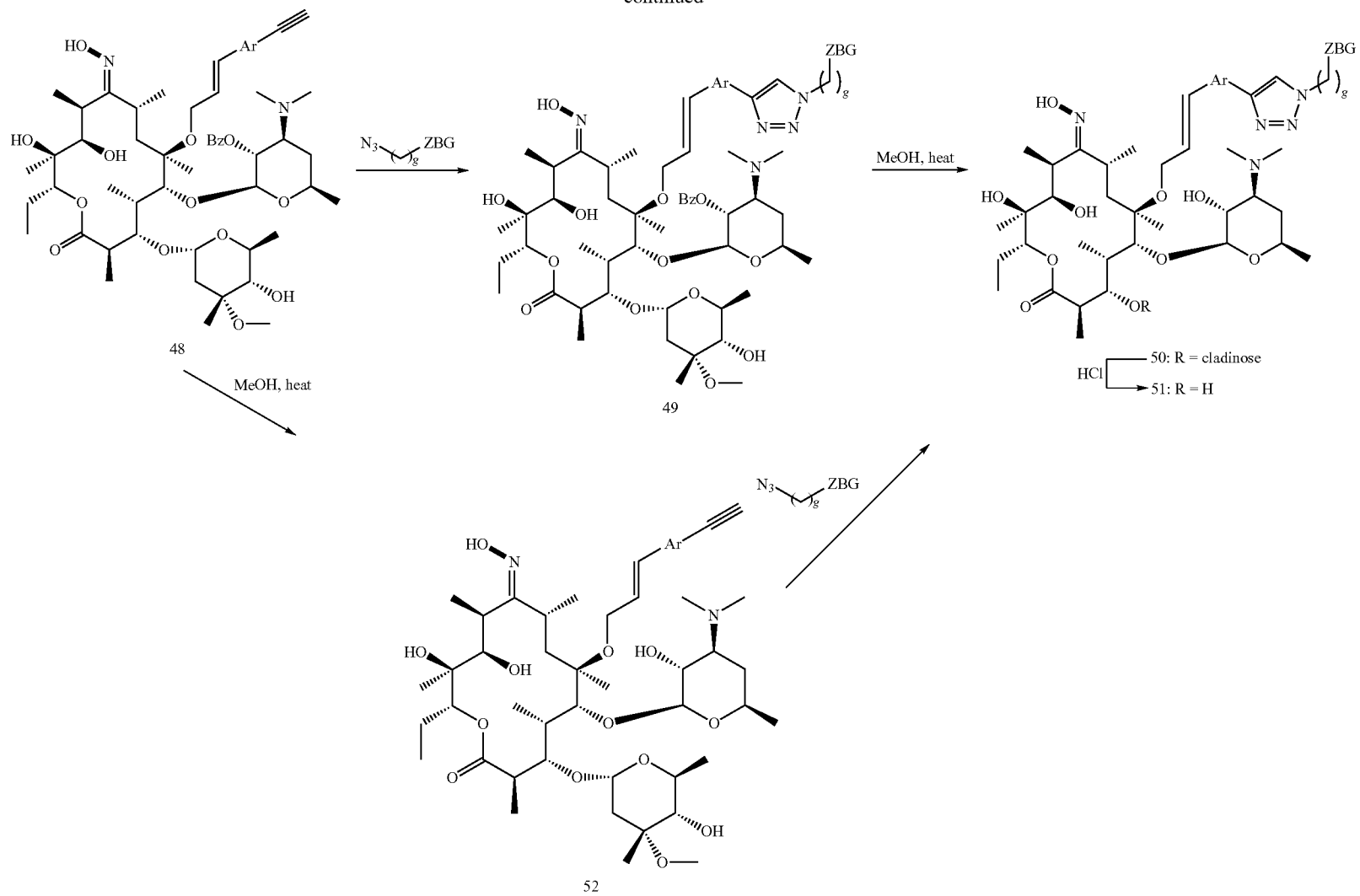
Scheme 4 illustrates representative general synthesis of triazole compounds with HDAC recognition cap-group connected to the macrocyclic ring at 06 position of 14-membered macrolide such as, but not limiting to, erythromycin. Adapting known protocols (see, Plata et al. (2004) *Tetra.*, 60: 10171), the aryl alkyne 47 can be obtained from readily available erythromycin A-9-oxime (see, Morimoto et al. (1990) *J. Antibiot.*, 43:286) through the intermediacy of 45.

**82**

Sequential base treatment with aqueous alkali and potassium carbonate in methanol will lead to alkyne 48 which can be reacted with azide 4 to give triazole 49. Methanolysis of 49 will afford triazole 50. Triazole 50 can be modified to form compound 51 by treatment with dilute mineral acid. Alternatively, methanolysis of alkyne 48 yields alkyne 51. Reaction of 51 with azide 4 will yield triazole 50.



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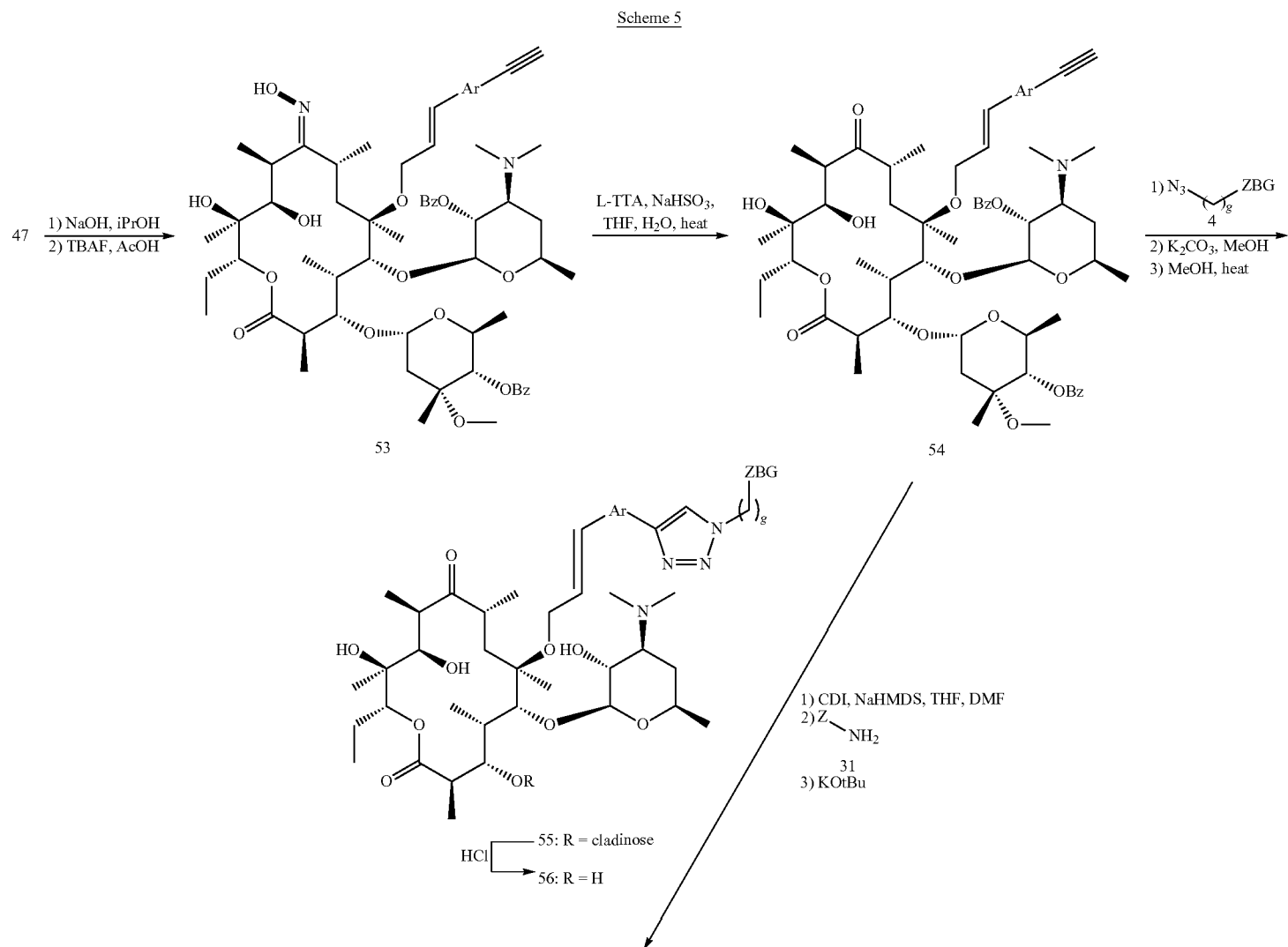
86

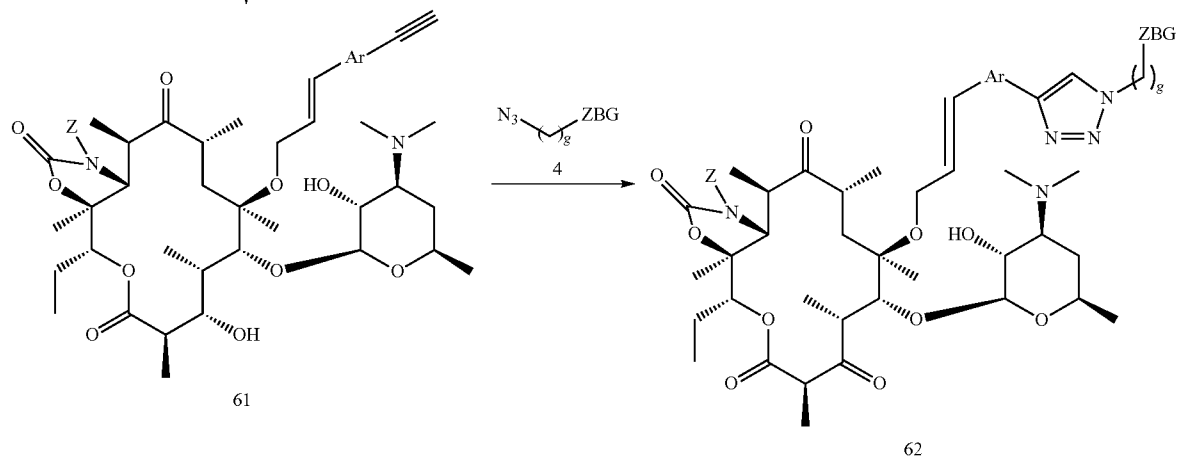
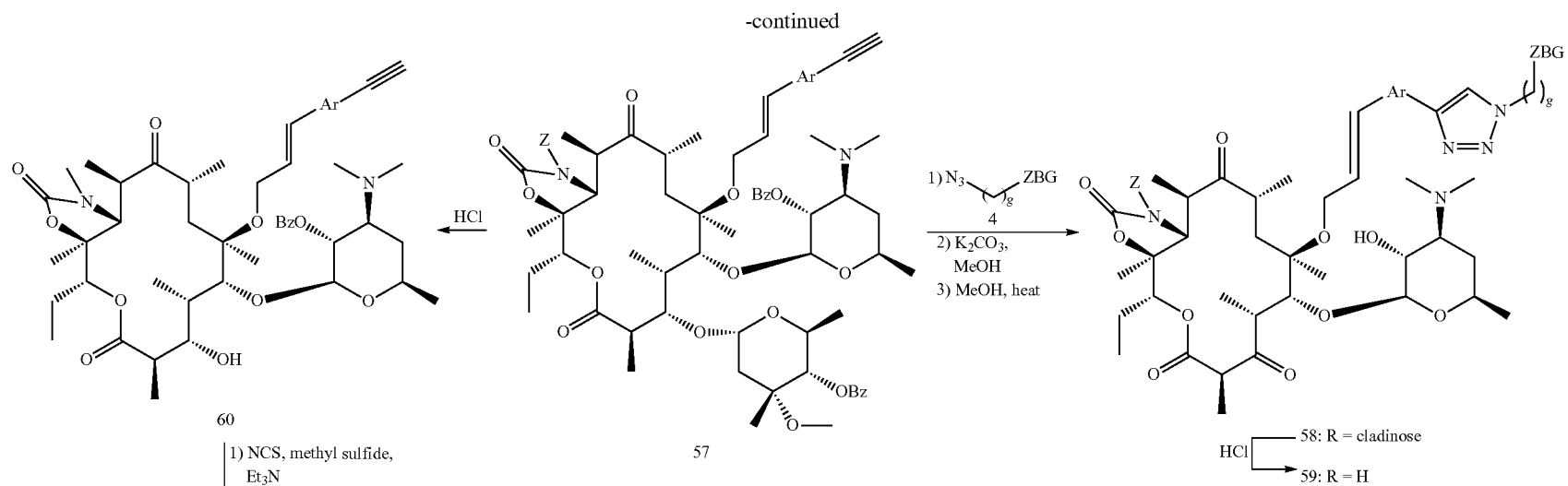
## 87

Scheme 5 illustrates representative general syntheses of triazole compounds with HDAC recognition cap-groups connected to the macrocyclic ring at the O6 position of 14-membered ketolides and carbamate modified macrolides. Selective benzoyl deprotection and silyl group removal will afford aryl alkyne 53. The oxime group in 53 can be removed by heating 53 in a THF/H<sub>2</sub>O containing NaHSO<sub>3</sub> and L-tartaric acid to afford aryl alkyne 54 (adapting protocols described by Plata et al. (2004) *Tetra.*, 60: 10171). Reaction of 54 with azide 4 followed by debenzoylation by sequential treatment with potassium carbonate in methanol and methanolysis at elevated temperatures will yield triazole 55. Triazole 55 can be modified to form compound 56 by treatment with dilute mineral acid. Aryl alkyne 54 can be modified to form aryl

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alkyne 57 by adapting procedures exemplified for similar transformations in scheme 3 or alternative protocols described in the art (see U.S. Pat. No. 5,631,355; Kashimura et al. (2003), *J. Antibiot.*, 56: 1062; Randolph et al. (2004) *J. Med. Chem.*, 47, 1085; Plata et al. (2004) *Tetra.*, 60: 10171). Reaction of 57 with azide 4 followed by debenzoylation by sequential treatment with potassium carbonate in methanol and methanolysis at elevated temperatures will yield triazole 58. Triazole 58 can be converted to compound 59 by treatment with dilute mineral acid. A direct treatment of 57 with dilute mineral acid will afford alcohol 60. Oxidation of 60 under Corey-Kim or similar conditions followed by methanolysis at elevated temperatures will yield alkyne ketolide 61. Reaction of 61 with azide 4 will yield triazole 62.



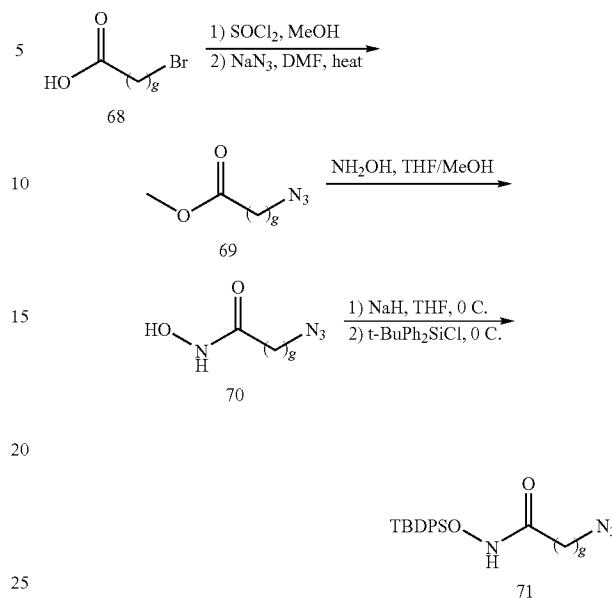


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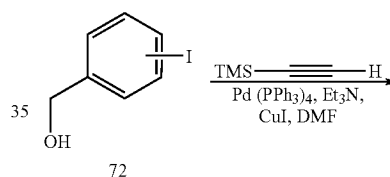
Intermediates such as electrophiles 2, 7 and 14, azide 4, nitrile oxide 11, amine 30 and carbonate alkyne 46 are all easily accessible by synthetic protocols known in the art. Exemplary examples for the synthesis of compounds whose general structures fit the forgoing are illustrated in schemes 6, 7, 8, and 9. Halogenated aryl alkyne 63 can undergo Heck coupling with ethyl acrylate to furnish  $\alpha,\beta$ -unsaturated ester 64. Reduction of 64 with DIBAL should generate alkenol 65, which can be transformed to carbonate 66 using methods known in the art (see U.S. Pat. No. 6,579,986; Plata et al. (2004), *Tetra.*, 60: 10171). Alternatively, alkenol 65 can be prepared from alkenol 67 through Hagihara-Sonogashira coupling (Belema et al. *Tet. Lett.*, (2004), 45: 1693). Reactions of carboxylic acid 68 with thionyl chloride in methanol follow by treatment with sodium azide should furnish azido methyl ester 69. Treatment of 69 with hydroxylamine should provide azido hydroxamate 70 (Ho et al., (2005), *J. Org. Chem.*, 70, 4873). The hydroxamate group of 70 can be appropriately protected with a silyl group to provide silyl azide 71 (Muri et al. *Org. Lett.*, (2000) 2: 539). Hagihara-Sonogashira coupling between alcohol 72 and TMS-acetylene should yield alkyne 73. TMS deprotection should furnish alkyne 74, which can be reacted with MsCl to provide mesylate 75. Reaction of phthalimide salt with 75 should provide phthalimide 76, which can be converted to amine 77 by hydrazinolysis or other suitable protocol known in the art. Treatment of alcohol 78, incorporating ZBG (appropriately protected when necessary), with oxidants such as PDC, should provide aldehyde 79. Reaction of 79 with hydroxylamine should furnish azide 80, which can be converted to nitrile oxide 81 by treatment with NBS or other reagents such as NCS, chloramine T, etc. Because of the likely instability of nitrile oxides, the generation of nitrile oxide can be performed in the presence of the appropriate alkyne 3 (scheme 1) to furnish the regioisomeric mixture of the isoxazoles.

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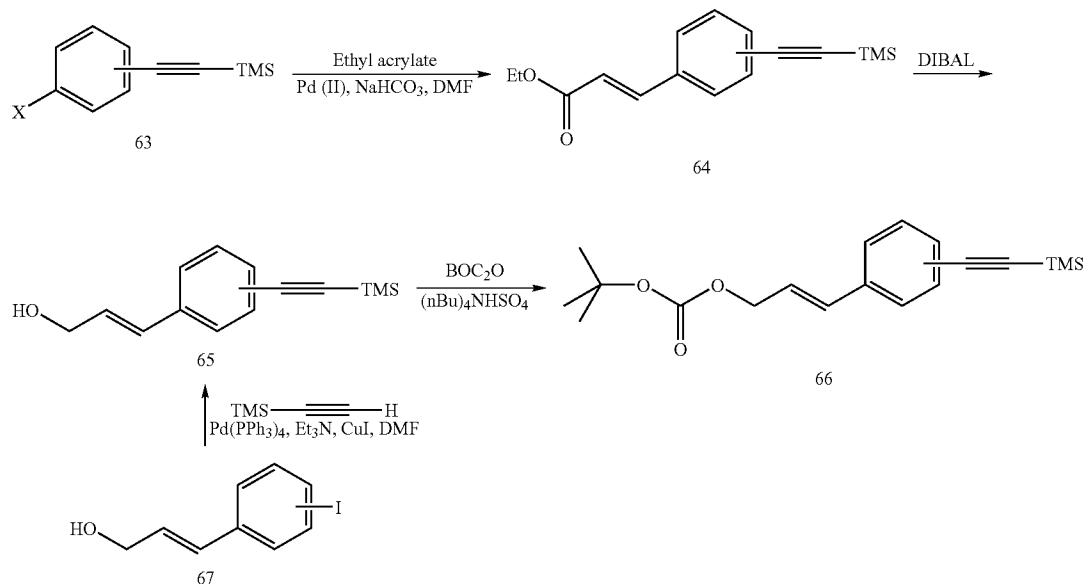
Scheme 7



Scheme 8



Scheme 6

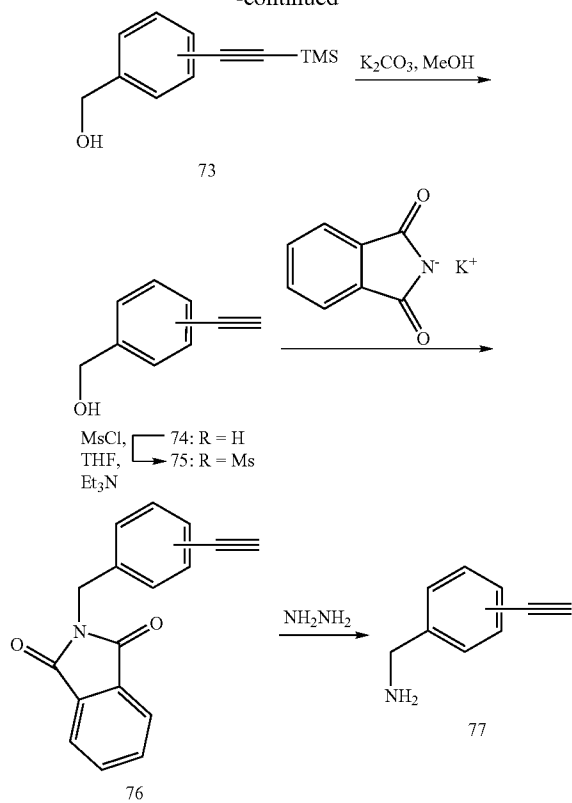


X = I, Br, OTf



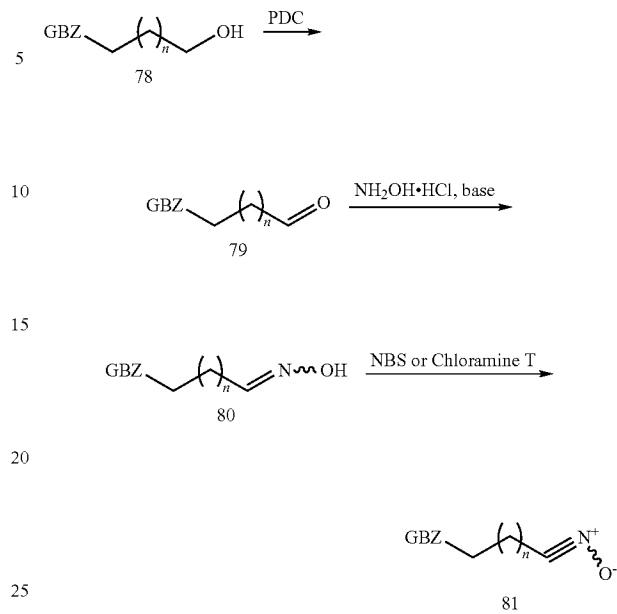
95

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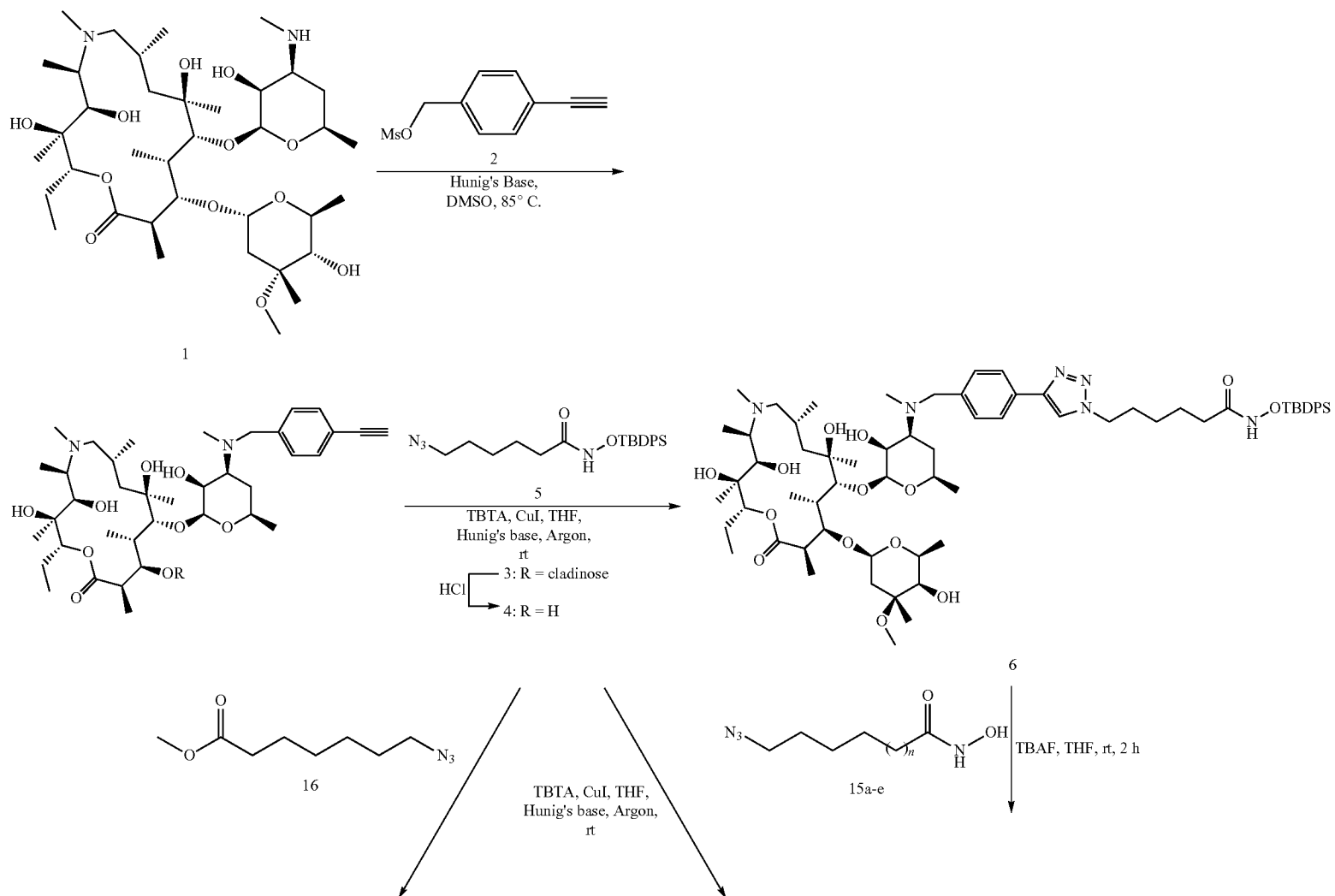
96

Scheme 9



Schemes 10 and 11 illustrate the synthesis of compounds 7-14 and 23-30 in Table 3.

Scheme 10: Synthesis of compounds 7-14

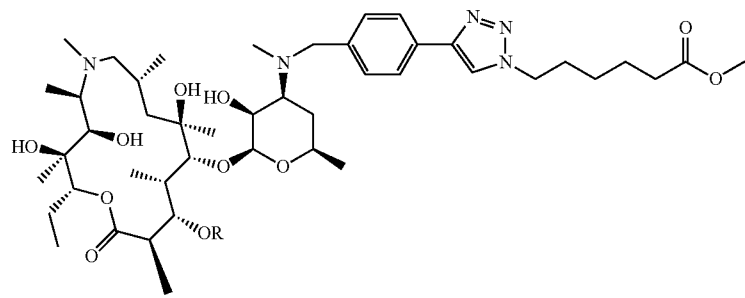


97

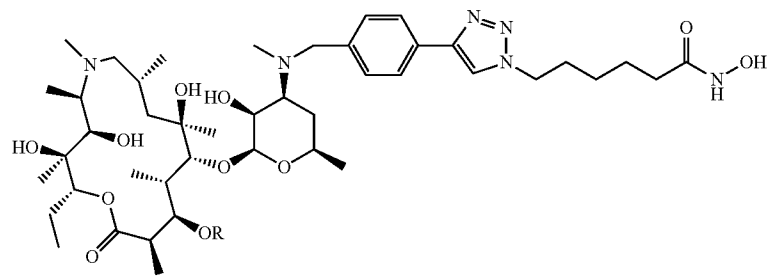
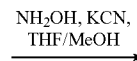
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17: R = cladinose  
18: R = H



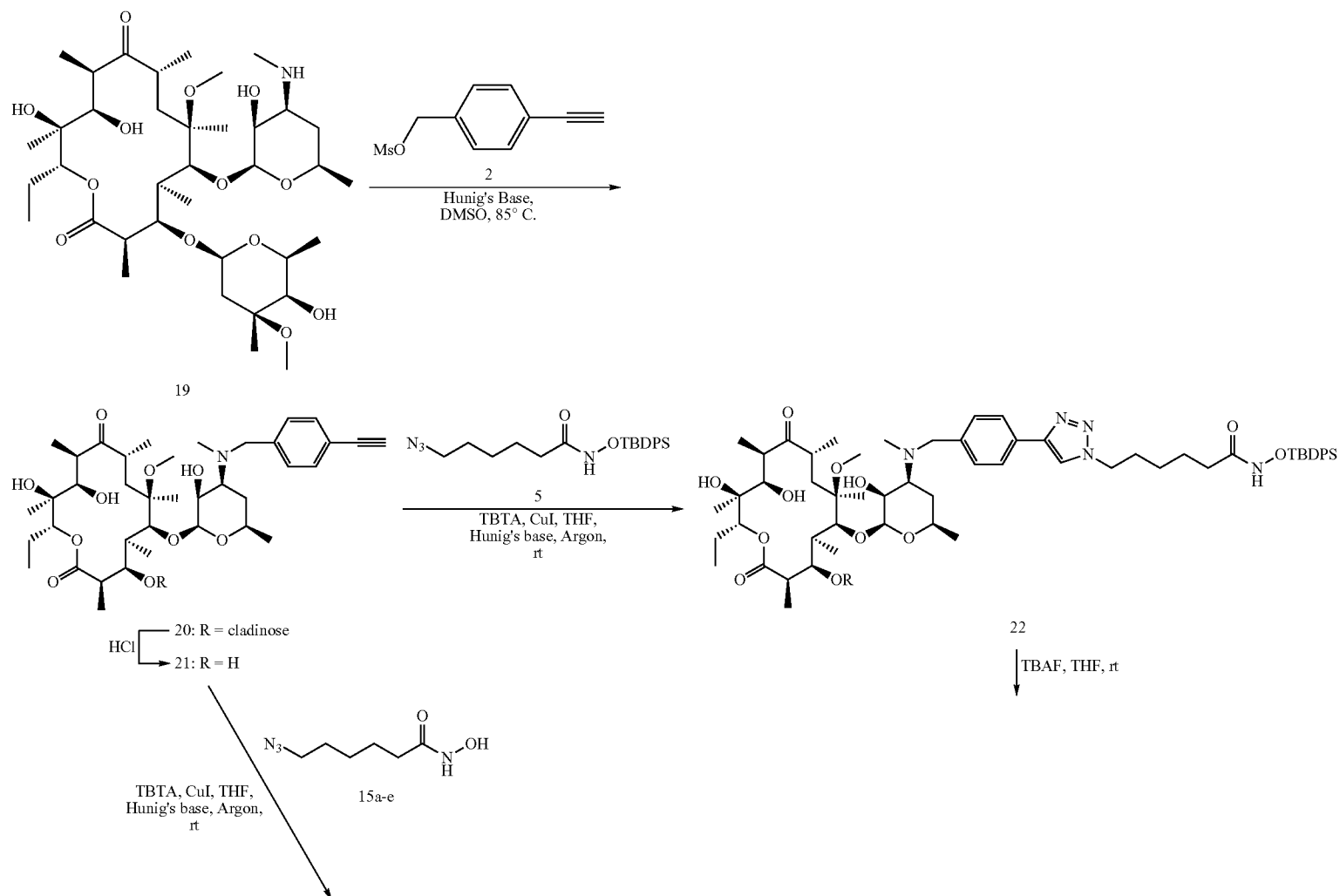
7, n = 1, R = cladinose  
8, n = 1, R = H  
9, n = 2, R = cladinose  
10, n = 2, R = H  
11, n = 2, R = cladinose  
12, n = 3, R = H  
13, n = 4, R = cladinose  
14, n = 5, R = cladinose

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Scheme 11: Synthesis of compounds 23-30

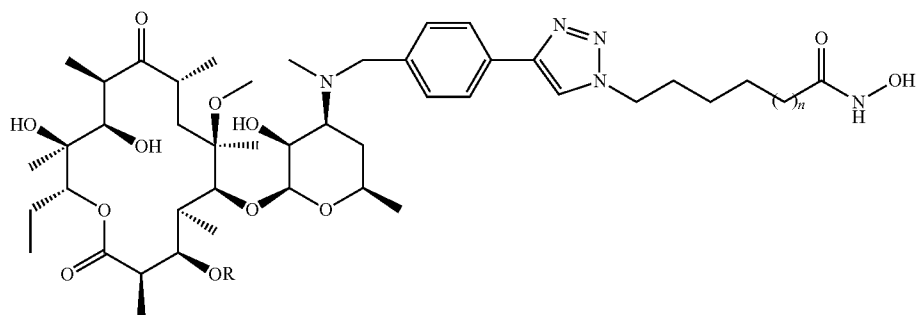


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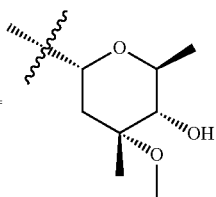
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-continued



- 23, n = 1, R = cladinoses
- 24, n = 1, R = H
- 25, n = 2, R = cladinoses
- 26, n = 2, R = H
- 27, n = 3, R = cladinoses
- 28, n = 3, R = H
- 29, n = 4, R = cladinoses
- 30, n = 5, R = cladinoses

cladinoses =



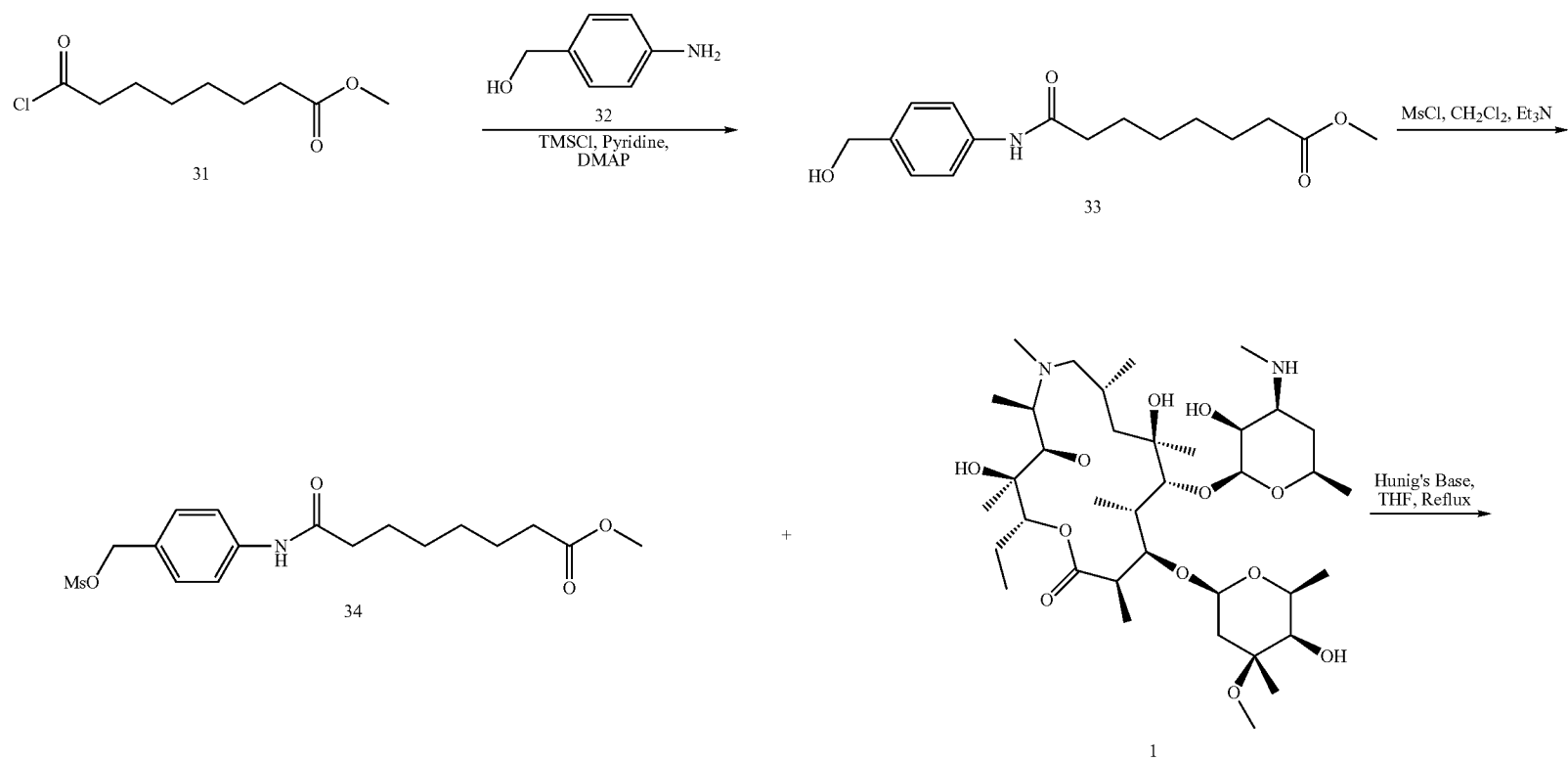
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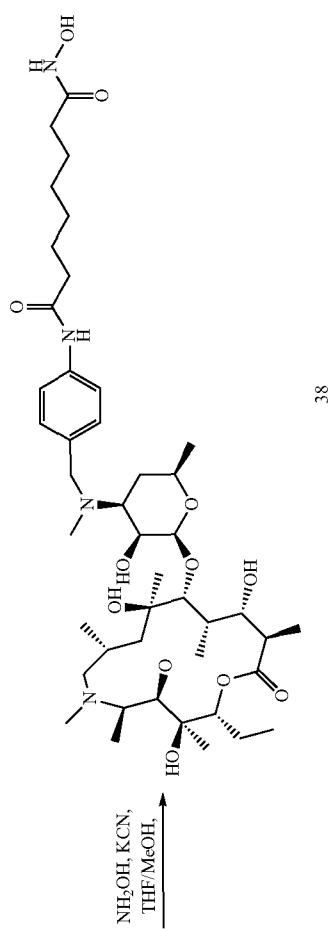
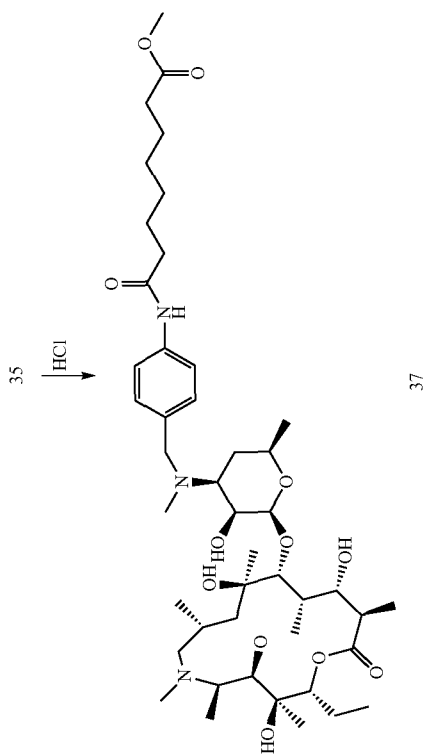
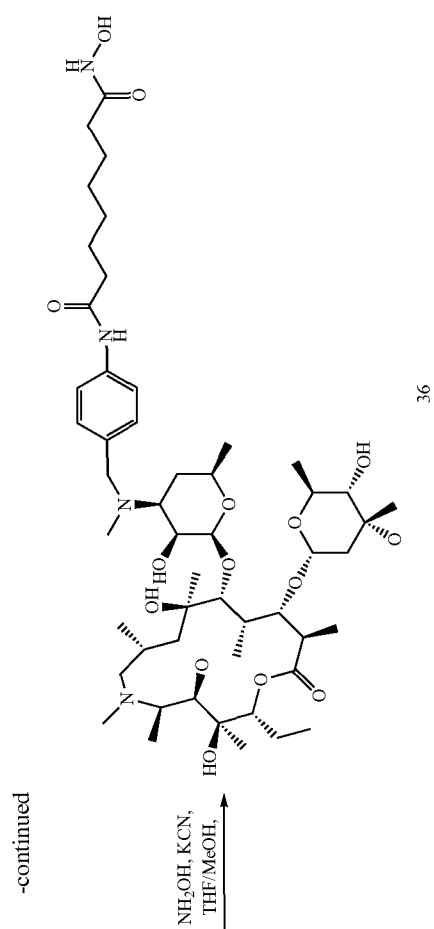
Scheme 12 illustrates the synthesis of compounds 36 and 38 in Table 3.

Scheme 12: Synthesis of compounds 36 and 38



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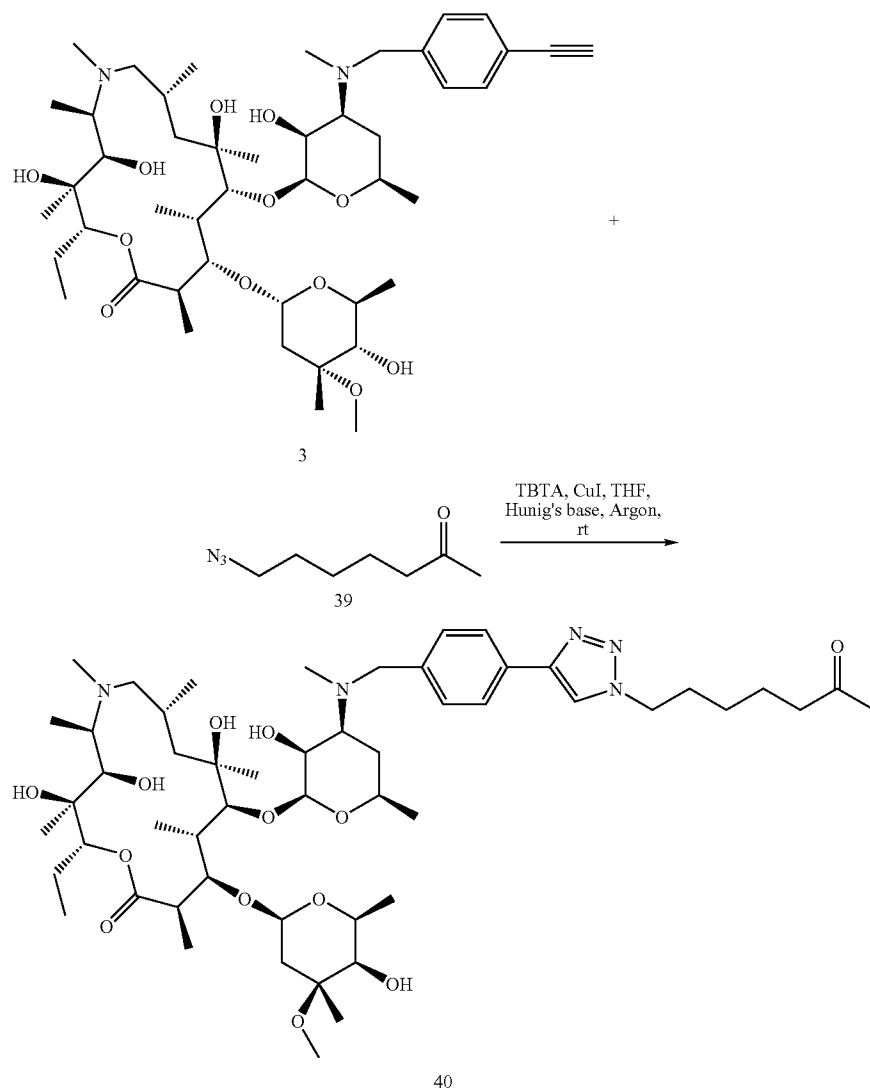
110



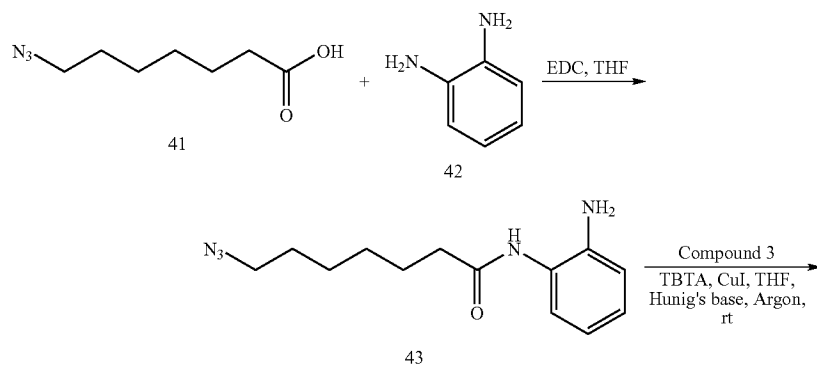


Schemes 13, 14 and 15 below illustrate the synthesis of compounds 40, 44 and 47 in Table 3.

Scheme 13: Synthesis of compound 40

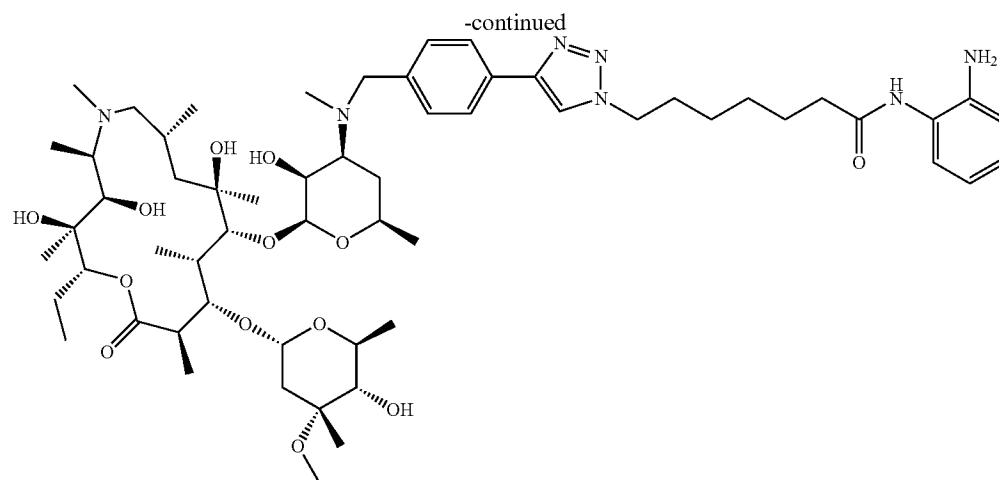


Scheme 14: Synthesis of compound 44



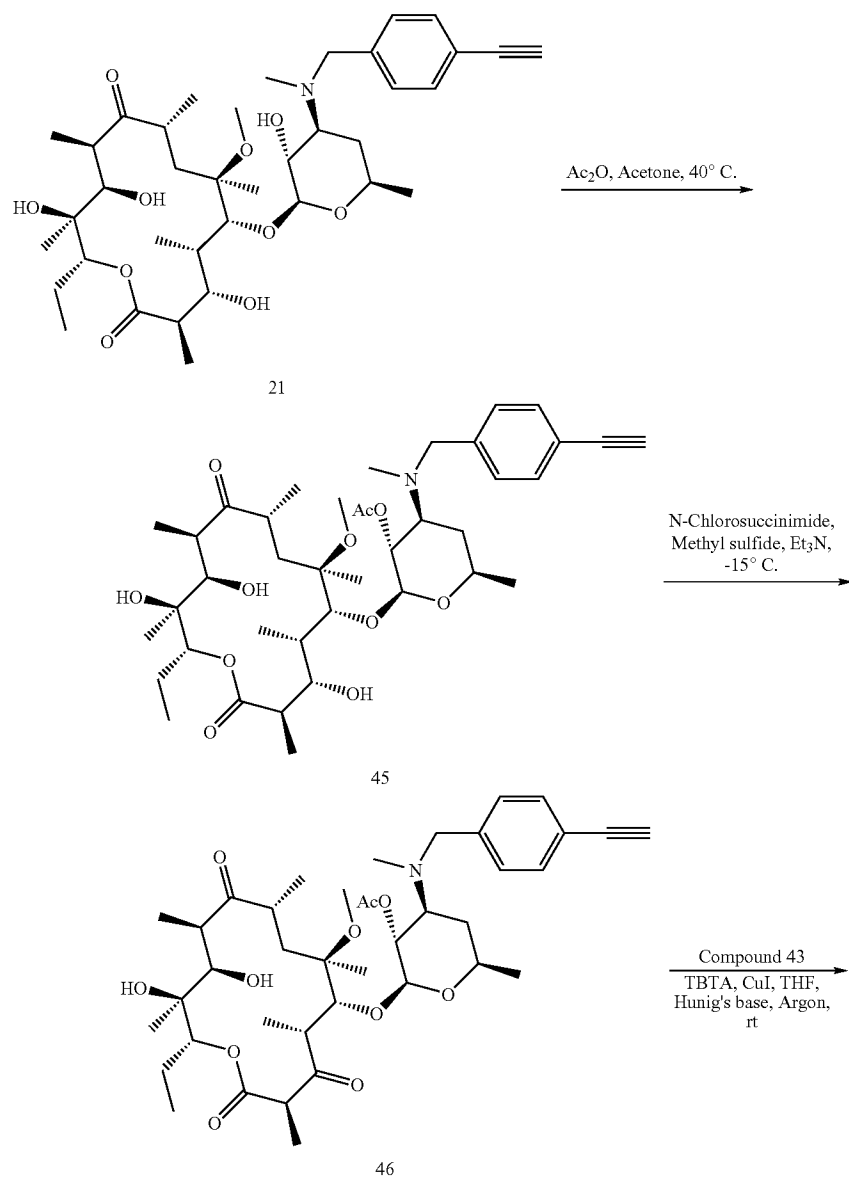
113

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44

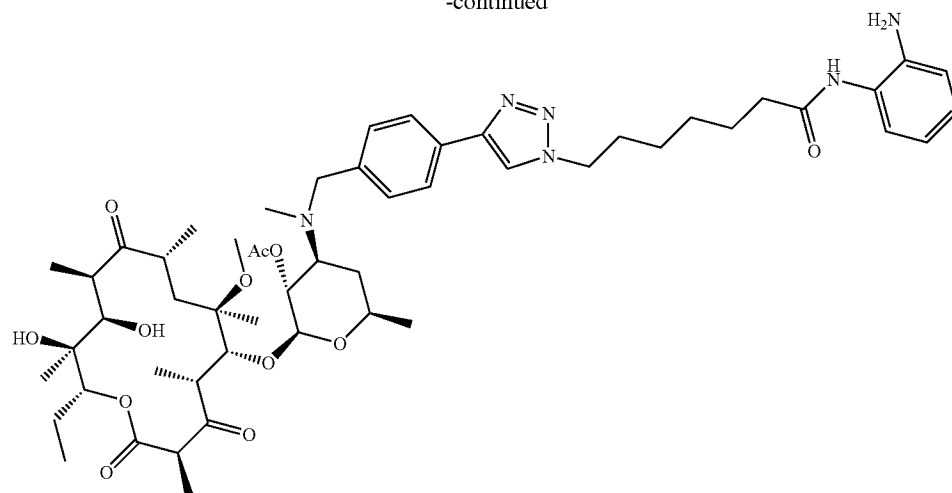
Scheme 15: Synthesis of compound 47



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47

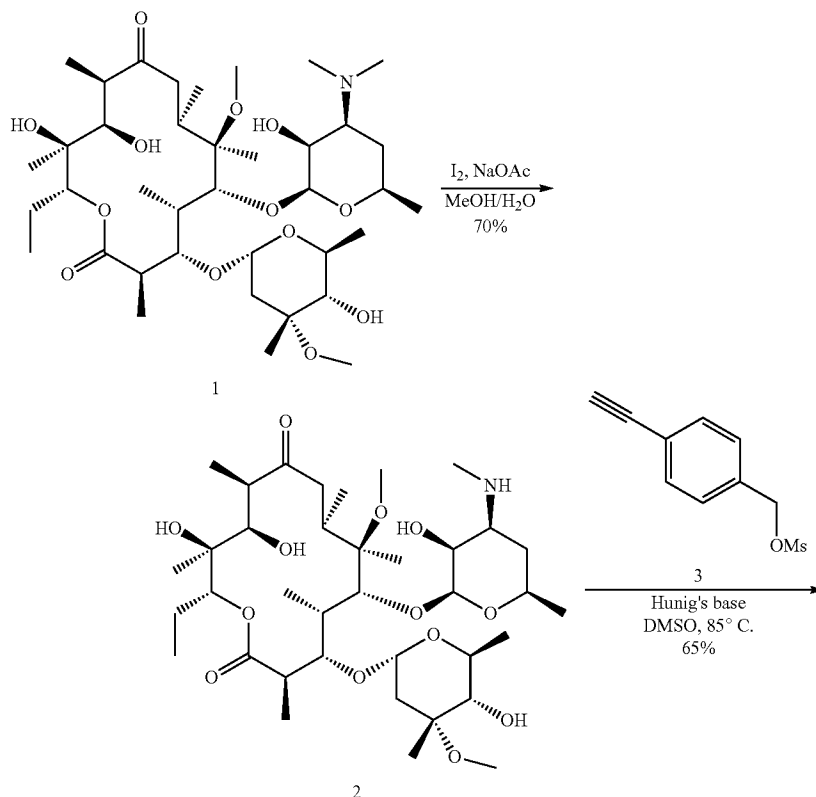
## Schemes 16A and 16B. Synthesis of Compound 56

Compound 56 can be synthesized via the 4'-ethynylbenzylketolide intermediate 9. Demethylation of clarithromycin 1 under standard conditions forms 4-desmethyl-clarithromycin 2 in 70% yield. Subsequent alkylation of 2 with 4-ethynylbenzyl methanesulfonate afforded the modified 4'-ethynylbenzylclarithromycin 4 which was treated with 1N HCl to selectively cleave the cladinose sugar to form compound 5. Reaction time for this step should be carefully controlled as longer reaction times led to the formation of significant amounts of byproduct. Selective acetylation of the 2'-OH group was accomplished by treating an acetone solution of 5 with acetic anhydride at 40 ° C. for 24 h to give compound 6 in 70% yield. Subsequent oxidation of the 3-hydroxyl group

of compound 6 to a 3-keto functional group, under anhydrous condition with NCS, afforded the ketolide 7 almost quantitatively. Treatment of 7 with excess carbonyldiimidazole (CDI) and NaHMDS in a mixture of THF/DMF afforded the 12-acyl imidazolidine 8 in 52% yield. Transformation into the desired tricyclic ketolide was achieved in two successive cyclization steps adapting literature procedures. Reaction of imidazolidine 8 with ethylenediamine followed by intramolecular Michael addition led to the formation of 11,12-cyclic carbamate. Subsequent intramolecular dehydration completed the cyclization process, affording the desired product 9 in 42% yield (Scheme 16A).

Scheme 16A. Synthesis of tricyclic ketolide from clarithromycin

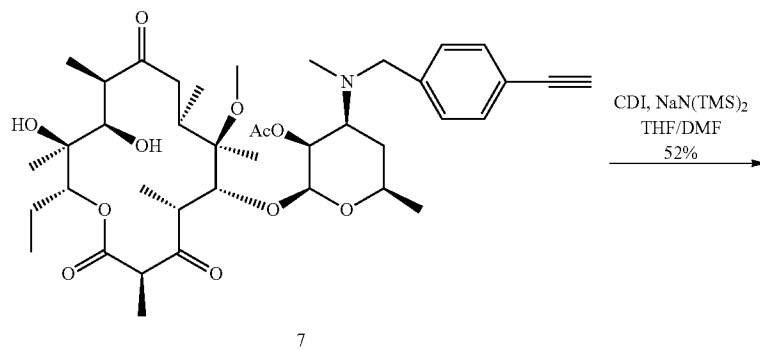
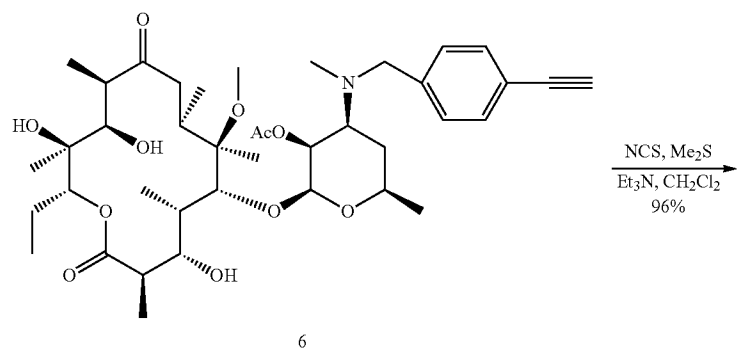
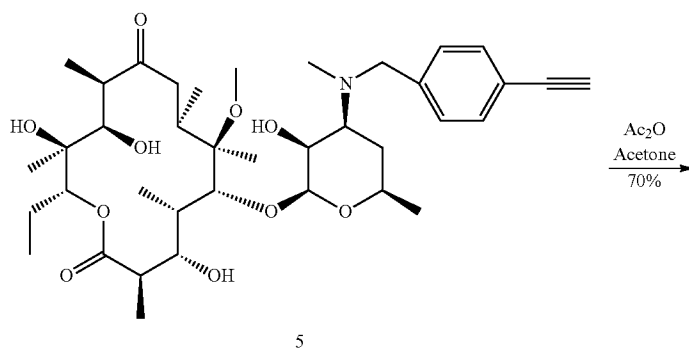
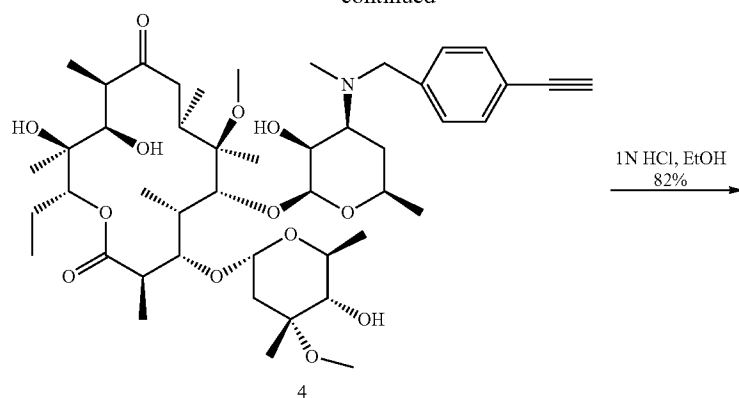
Scheme 16A. Synthesis of tricyclic ketolide from clarithromycin



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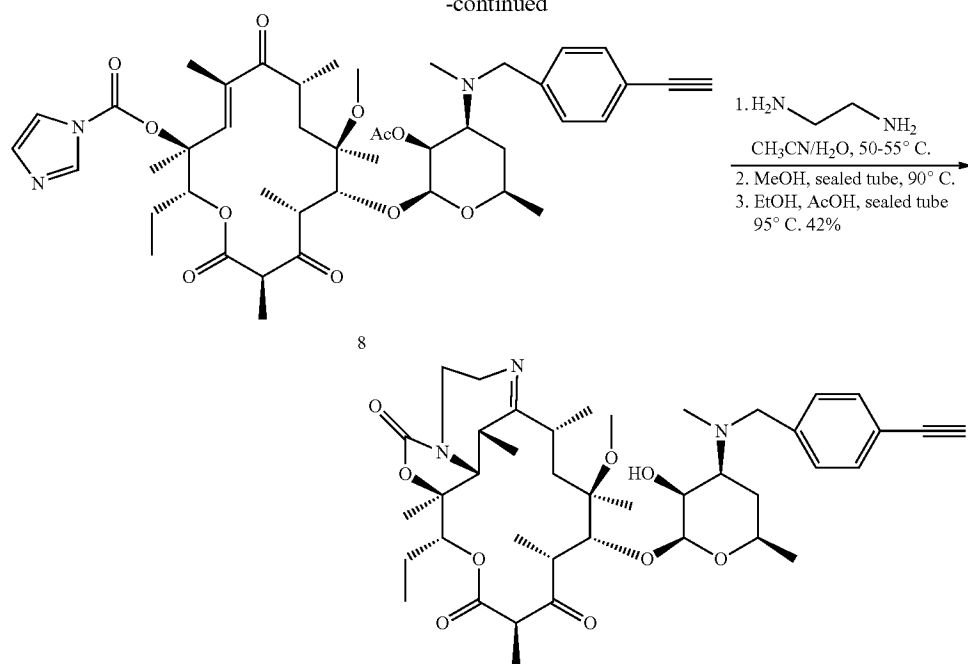
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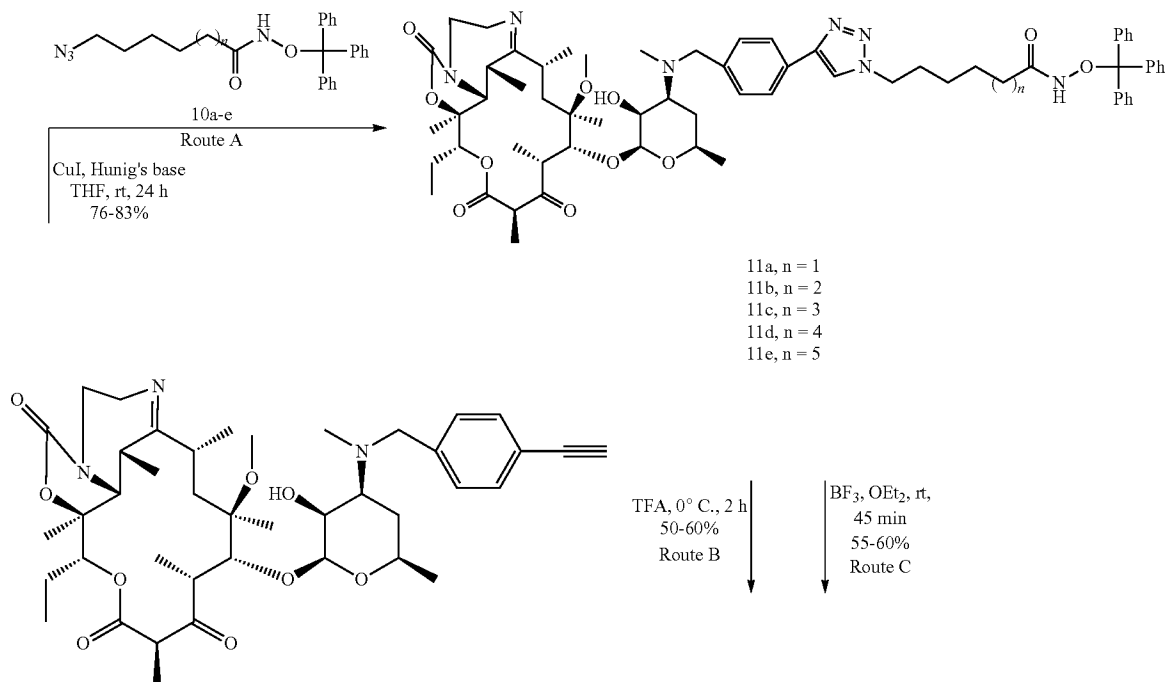


9

Copper (I) catalyzed cycloaddition of 9 with O-trityl protected azido hydroxamate analogs 10a-e (route A), afforded the 1,2,3-triazole linked derivatives 11a-e. Deprotection of the trityl group in 11a-e with TFA (route B) gave the desired products (56a-e) in good yields. A similar outcome was obtained when trityl group deprotection was effected with

$\text{BF}_3 \cdot \text{OEt}_2$  (route C). Additionally, the desired hydroxamates could be obtained through direct copper (I) catalyzed cycloaddition between unprotected azido hydroxamate 13 and alkyne 9 (route D). Scheme 16B. Routes for the preparation of compound 56 from compound 9.

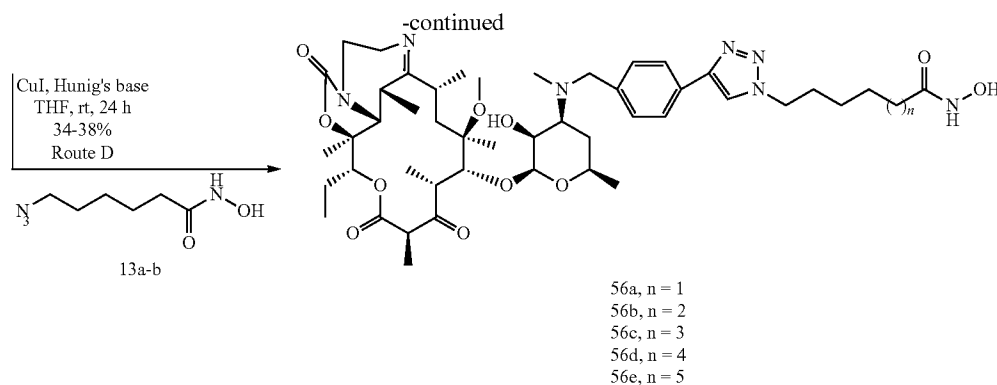
Scheme 16 B. Routes for the preparation of compounds 56 from compound 9.



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## V. Methods of Use

The compounds described herein may be used as anti-cancer agents, anti-inflammatory agents, anti-infective agents, anti-malarial agents, cytoprotective agents, neuroprotective agents, chemopreventive agents, prokinetic agents, and/or cognitive enhancing agents. Examples of cancer which may be treated include, but are not limited to, epithelial cancers, such as melanoma, lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, colon cancer, and bladder cancer; hematological cancers, such as multiple myeloma, leukemia, and lymphoma; cervical cancer; and liver cancer.

The potency of the compounds described herein were investigated by determining the drug concentrations necessary for 50% inhibition of cell viability ( $IC_{50}$ ) in SKMES 1, NCI-H69, DU 145 cells, lung fibroblasts, and HMEC. Drug concentrations necessary for 50% inhibition of cell viability ( $EC_{50}$ ) were quantitatively measured using trypan blue exclusion according to literature protocol. Table 5 in the examples shows the  $EC_{50}$  values for each compound. All compounds inhibited the proliferation of the transformed cells studied with  $EC_{50}$  in the low micromolar range. Most importantly, the compounds were less toxic to untransformed cell-lines (lung fibroblast and HMEC).

The compounds described herein were also evaluated for parasitic activity against *Plasmodium falciparum* and *Leishmania donovani*. *P. falciparum* and *L. donovani* are the causative parasites of malaria and leishmaniasis, two human diseases which constitute a serious threat to public health in tropical and sub-tropical countries. Antimalarial activity was evaluated in vitro using chloroquine-sensitive (D6, Sierra Leone) and chloroquine-resistant (W2, Indochina) strains of *P. falciparum*. Antileishmanial activity was evaluated in vitro against the promastigote stage of *L. donovani*.

*Plasmodium* growth inhibition was determined by a parasite lactate dehydrogenase assay using Malstat reagent. Inhibition of viability of the promastigote stage of *L. donovani* was determined using standard Alamar blue assay, modified to a fluorometric assay. Amphotericin B and pentamidine, standard antileishmanial agents; chloroquine and artemisinin, standard antimalarial; and suberoylanilide hydroxamic acid (SAHA), standard HDACi were all used as positive controls. To determine selective toxicity index, all compounds were tested against nontransformed mammalian cell lines namely, monkey kidney fibroblasts (Vero) and murine macrophages (J774.1) using Neutral Red assay.

The non-peptide macrocyclic HDAC inhibitors potently inhibited the proliferation of both the sensitive and resistant strains of *P. falciparum* with an  $IC_{50}$  ranging from 0.1  $\mu$ g/mL to 3.5  $\mu$ g/mL. In particular, compounds 5-8 in Table 16,

derived from either the 14- or 15-membered macrocyclic analogs and having 6 methylene spacers separating the triazole ring from the zinc-binding hydroxamic acid group ( $n=6$ ), had the most potent antimalarial activities in this series. These compounds are equipotent or >4-fold more potent than the control compound SAHA. Moreover, they are several folds more selectively toxic to either strains of *P. falciparum* compared to SAHA.

The antimalarial activities of these macrocyclic HDACi followed a similar trend as their anti-HDAC activity against HDAC 1/2 from HeLa nuclear extract, suggesting that parasite HDACs could be an intracellular target of these compounds.

Compounds 5 and 9 exhibited modest activity against the promastigote stage of *L. donovani*. A comparison of the antileishmanial activities of compounds 13 and 14, analogs with  $n=8$ , revealed a disparity between the activity of 14- and 15-membered macrocyclic rings. 14-Membered compound 13 is 5- to 6-fold more potent than its 15-membered congener 14. However, this disparity dissipates after a single methylene group extension ( $n=9$ ), as compounds 15 and 16 have virtually indistinguishable antileishmanial activities. Comparatively, compounds 13, 15 and 16, analogs with the most potent antileishmanial activities, are about 7- to 10-fold more potent than SAHA and approximately 3-fold less potent than pentamidine.

Since these non-peptide macrocyclic HDACi have nanomolar anti-HDAC activities, the observed disparity in the trend of their antimalarial and antileishmanial activities may have implication in the organization of the active sites of the relevant *P. falciparum* and *L. donovani* HDAC isozymes. These observations provide additional evidence of the suitability of HDAC inhibition as a viable therapeutic option to curb infections caused by apicomplexan protozoans and trypanosomatids (1, 5, 6, 14) and could facilitate the identification of other HDACi that are more selective for either parasite.

The formulations contain an effective amount of one or more HDAC inhibitors. The doses in which the HDAC inhibitors and their salts, prodrugs, or solvates can be administered may vary widely depending on the condition of the patient and the symptoms to be treated. One of ordinary skill in the art can readily determine the necessary dosage based on the condition of the patient and the disease to be treated.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

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Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

## EXAMPLES

## Materials

“Preparative TLC” or “prep TLC” refers to preparative thin layer chromatography and was performed on Analtech preparative TLC plates (UV 254, 2000  $\mu$ m), unless otherwise stated. “Column chromatography” or “flash column chromatography” was performed with 200-400 Mesh silica gel, unless otherwise noted.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer.  $^1\text{H}$  NMR spectra were recorded in parts per million (ppm) relative to the peak of  $\text{CDCl}_3$ , (7.24 ppm),  $\text{CD}_3\text{OD}$  (3.31 ppm), or  $\text{DMSO}-d_6$  (2.49 ppm).  $^{13}\text{C}$  spectra were recorded relative to the central peak of the  $\text{CDCl}_3$  triplet (77.0 ppm),  $\text{CD}_3\text{OD}$  (49.0 ppm), or the  $\text{DMSO}-d_6$  septet (39.7 ppm), and were recorded with complete hetero-decoupling.

Common reaction solvents were either high performance liquid chromatography (HPLC) grade or American Chemical Society (ACS) grade, and used without further purification. Anhydrous solvents and other reagents were purchased and used without further purification.

Fluor de Lys<sup>TM</sup> is a fluorescence based HDAC activity assay comprising a combination of fluorogenic Histone deAcetylase Lysyl substrate and a developer. The kit is a highly sensitive and convenient alternative to radiolabeled, acetylated histones or peptide/HPLC methods for the assay of histone deacetylases. This assay is based on the ability of HeLa nuclear extract, which is enriched in HDAC activity, to mediate the deacetylation of the acetylated lysine side chain of the Fluor de Lys substrate. The assay procedure requires two steps. First, incubation of the HeLa nuclear extract with the Fluor de Lys substrate results in substrate deacetylation and thus sensitizes it to the second step. In the second step, treatment of the deacetylated substrate with the Fluor de Lys developer produces a fluorophore. The substrate-developer reaction, under normal circumstances goes to completion in less than 1 min at 25° C. The kit used was the Fluorimetric Assay/Drug Discovery Kit—AK-500 Manual Fluorescent Assay System available from BIOMOL® International, Plymouth Meeting, Pa.

The numbers used to identify the compounds described in the examples correspond to the references numbers in Table 3 and/or reaction schemes 10-15.

## Example 1

## Synthesis of Compounds 7-14 and 23-30 in Table 3

## Synthesis of Azithromycin-N-phenylacetylene (3)

To a solution of N-demethylated azithromycin 1 (2.0 g, 2.56 mmol) in anhydrous DMSO (30 ml) was added Hunig's base (4 ml) and 4-ethynylbenzyl methanesulfonate 2 (0.760 g, 3.60 mmol). The reaction mixture was heated with stirring under argon at 85° C. for 2.5 h. The reaction was cooled and diluted with ethyl acetate (EtOAc, 100 mL) and washed with saturated  $\text{NaHCO}_3$  (3 $\times$ 60 mL) and saturated brine (60 mL). The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo. The crude product was purified by flash chromatogra-

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phy (silica, 12:1:0.05  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{conc. NH}_4\text{OH}$ ) to give 1.2 g (52%) of 3 as a brownish white solid.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.84 (m), 0.97 (d, J=7.6 Hz), 1.04 (m), 1.12-1.32 (m), 1.36-1.53 (m), 1.66-1.75 (m), 1.81-2.07 (m), 2.19 (s), 2.25-2.29 (m), 2.48 (m), 2.63-2.73 (m), 2.89 (bs), 2.96 (t, J=9.8 Hz), 3.02 (s), 3.08 (s), 3.27-3.32 (m), 3.38-3.45 (m), 3.56 (d, J=6.8 Hz), 3.63 (s), 3.72 (d, J=13.2 Hz), 3.97 (m), 4.19 (m), 4.36 (d, J=7.2 Hz), 4.63 (d, J=10 Hz), 5.04 (d, J=4.4 Hz), 7.21 (d, J=8 Hz), 7.39 (d, J=8 Hz);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  7.5, 9.2, 11.3, 14.9, 16.3, 18.3, 21.3, 21.4, 21.6, 22.0, 26.8, 27.6, 29.6, 34.7, 36.3, 36.9, 42.0, 42.3, 45.2, 49.2, 57.7, 62.3, 63.7, 65.4, 68.5, 70.0, 70.6, 72.7, 73.5, 73.8, 74.2, 77.1, 77.9, 78.0, 83.4, 83.7, 94.5, 102.6, 120.8, 128.5, 132.0, 139.6, 178.3; HRMS (FAB, m/ba) calc for  $[\text{C}_{46}\text{H}_{76}\text{N}_2\text{O}_{12} + \text{H}]^+$  849.5476. found 849.5411.

## Synthesis of Azithromycin-arylalkyltriazolyl methyl ester (17)

Azithromycin-N-phenylacetylene 3 (0.045 g, 0.053 mmol) and azido-ester 16 (0.014 g, 0.080 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.053 mmol), and Hunig's base (0.05 mL) were then added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (30 mL) and washed with 1:4  $\text{NH}_4\text{OH}/\text{saturated NH}_4\text{Cl}$  (3 $\times$ 25 mL) and again with saturated  $\text{NH}_4\text{Cl}$  (25 mL). The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with Hexane/EtOAc/ $\text{Et}_3\text{N}$  3:2:0.1 to give 50 mg (92%) of 17 as a white-brown solid.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.82-0.90 (m), 0.98 (d, J=7.6 Hz), 1.05-1.13 (m), 1.19-1.23 (m), 1.25-1.30 (m), 1.40-1.52 (m), 1.60-1.74 (m), 1.80-1.96 (m), 2.00-2.06 (m), 2.22-2.37 (m), 2.56 (m), 2.67 (m), 2.95 (t, J=9.8 Hz), 3.07 (s), 3.29-3.34 (m), 3.46 (bs), 3.54 (d, J=6.8 Hz), 3.61 (s), 3.68 (bs), 3.77 (m), 3.97 (m), 4.18 (m), 4.34-4.38 (m), 4.69 (m), 5.06 (d, J=4 Hz), 7.32 (d, J=6.4 Hz), 7.73-7.75 (m);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  8.7, 9.2, 11.3, 14.2, 14.7, 16.5, 18.2, 21.4, 21.5, 22.2, 24.2, 25.9, 26.6, 27.3, 29.7, 30.0, 33.6, 34.6, 36.4, 36.9, 42.4, 45.3, 45.8, 49.3, 50.0, 51.5, 57.7, 63.9, 65.5, 68.6, 69.4, 70.5, 72.7, 73.8, 74.2, 77.2, 77.6, 78.0, 83.4, 94.4, 102.7, 119.3, 125.5, 129.1, 129.4, 147.2, 173.4, 178.1. MS (FAB, m/ba) 1020.3 (M+H)<sup>+</sup>.

## Synthesis of

## Descladinoseazithromycin-arylalkyltriazolyl methyl ester (18)

A mixture of compound 3 (0.12 g, 0.14 mmol) in 0.25 N HCl (15 mL) was stirred at room temperature for 20 h and poured into EtOAc (20 mL). The two layers were separated and the aqueous layer was washed with EtOAc (2 $\times$ 20 mL), basified with concentrated  $\text{NH}_4\text{OH}$  and then extracted with 5% MeOH in  $\text{CH}_2\text{Cl}_2$  (2 $\times$ 30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . Solvent was evaporated off to give 89 mg (91%) of descladinose compound 4 as a white solid.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.81-0.87 (m), 0.90-1.07 (m), 1.17-1.27 (m), 1.31-1.59 (m), 1.68-1.71 (m), 1.80-1.87 (m), 1.99-2.03 (m), 2.08 (s), 2.23-2.27 (m), 2.31 (s), 2.44-2.48 (m), 2.59-2.73 (m), 3.32-3.39 (m), 3.48-3.53 (m), 3.60-3.65 (m), 3.73 (d, J=9.6 Hz), 3.84-3.91 (m), 4.43 (d, J=7.2 Hz), 4.69-4.72 (m), 7.16 (d, J=8.0 Hz), 7.39 (d, J=8.4 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  7.7, 7.9, 10.9, 14.2, 16.1, 20.9, 21.1, 21.2, 25.9, 26.6, 29.3, 36.0, 36.4, 37.1, 42.1, 44.5, 57.6, 60.3, 62.5, 65.3, 69.9,

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70.5, 70.9, 73.0, 74.1, 75.4, 77.2, 79.5, 83.3, 94.9, 106.5, 120.8, 128.3, 132.0, 139.3, 177.2. MS (FAB, m/ba) 691.2 (M+H)<sup>+</sup>.

The descladinose compound 4 (0.080 g, 0.115 mmol) and azido-ester 16 (0.030 g, 0.173 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.053 mmol), and Hunig's base (0.05 mL) were then added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (3×25 mL) and again with saturated NH<sub>4</sub>Cl (25 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by preparative TLC, eluting with Hexane/EtOAc/Et<sub>3</sub>N 3:2:0.1 to give 65 mg (65%) of 18 as a white-brown solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.79-0.86 (m), 1.00-1.07 (m), 1.17-1.35 (m), 1.42-1.51 (m), 1.55-1.72 (m), 1.80-1.94 (m), 2.00-2.05 (m), 2.1 (s), 2.23-2.27 (m), 2.33 (s), 2.47 (d, J=10.4 Hz), 2.58-2.72 (m), 3.32-3.41 (m), 3.52-3.73 (m), 3.92-4.00 (m), 4.34 (t, J=7.0 Hz), 4.41 (d, J=7.6 Hz), 4.69 (d, J=10.8 Hz), 7.24 (d, J=8.4 Hz), 7.71 (d, J=8 Hz), 7.73 (s); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 7.7, 7.9, 8.7, 10.9, 16.1, 20.9, 21.2, 24.2, 25.8, 25.9, 26.6, 29.2, 29.6, 30.0, 33.6, 36.0, 36.3, 37.1, 42.0, 44.5, 45.8, 50.0, 51.5, 57.7, 62.6, 65.1, 69.9, 70.4, 73.1, 74.1, 75.3, 79.4, 94.8, 106.4, 119.3, 125.5, 128.9, 129.6, 138.2, 147.1, 173.4, 177.2. MS (FAB, m/ba) 862.2 (M+H)<sup>+</sup>.

Synthesis of  
Azithromycin-N-phenyltriazolylhexahydroxamic  
acid (7)

Method A

To a solution of compound 17 (0.04 g, 0.04 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H<sub>2</sub>O) (0.03 mL, 0.54 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and saturated sodium bicarbonate (25 mL), the two layers were separated and the aqueous layer was extracted with 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (2×20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH 10:1:0.1 to give compound 7 (6.5 mg, 16%) as brown-white solid.

Method B

Azithromycin-N-phenylacetylene 3 (0.100 g, 0.109 mmol) and 6-azido-hexahydroxamic acid 15a (0.081 g, 0.117 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper (I) iodide (0.011 g, 0.07 mmol) and Hunig's base (0.5 mL) were then added to the reaction mixture, and stirring continued for 4 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (3×30 mL) and saturated NH<sub>4</sub>Cl (30 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) to give 71 mg (59%) of 7 as a brownish white solid.

Method C

Azithromycin-N-phenylacetylene 3 (0.045 g, 0.050 mmol) and 6-azido-O-silyl hexahydroxamate 6 (0.060 g, 0.146 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature (Note: compound 6 was prepared from the corresponding azido carboxylic acid, t-BuPh<sub>2</sub>SiCl and NaH, according to the procedure described by Muri et al. ORG. LETT (2000) 2: 539). Copper (I) iodide (0.010 g, 0.05 mmol), Hunig's base (0.5 mL) and tris[(1-

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benzyl-1H-1,2,3-triazol-4-yl)methyl]amine, (TBTA) (0.016 g, 0.030 mmol) were then added to the reaction mixture, and stirring continued for 2 h (Note: TBTA was synthesized according to Chen et al. Org. Lett., (2004) 6: 2853). The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (2×30 mL) and saturated NH<sub>4</sub>Cl (30 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) to give 38 mg (60%) of silyl protected compound 6 as a brownish white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.82-0.92 (m), 1.00-1.14 (m), 1.17-1.27 (m), 1.30-1.32 (m), 1.35-1.59 (m), 1.73-1.92 (m), 2.01-2.15 (m), 2.24-2.32 (m), 2.36 (br s), 2.56 (d, J=10.4 Hz), 2.69 (m), 2.98 (d, J=10 Hz), 3.07-3.09 (m), 3.32-3.36 (m), 3.42-3.46 (m), 3.55-3.63 (m), 3.66 (s), 3.78 (d, J=13.2 Hz), 4.01 (m), 4.15-4.25 (m), 4.40 (d, J=6.8 Hz), 4.63 (d, J=7.2 Hz), 4.69 (s), 5.10 (d, J=4.4 Hz), 7.31-7.43 (m), 7.65-7.75 (m).

To a solution of silyl protected compound 6 (0.025 g, 0.02 mmol) in THF (1 mL) was added 1 M TBAF in THF (0.030 mL, 0.030 mmol) and the mixture was stirred at room temperature for 2 h during which TLC revealed a near quantitative conversion to a lower Rf product. The reaction was partitioned between CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and saturated NH<sub>4</sub>Cl (25 mL), the two layers were separated and the organic layer dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/Et<sub>3</sub>N) to give 15 mg (73%) of 7 as a brownish white solid.

<sup>1</sup>H-NMR (Acetone-d<sub>6</sub>, 400 MHz) δ 0.83-0.92 (m), 1.02 (d, J=7.6 Hz), 1.08-1.11 (m), 1.14 (d, J=7.6 Hz), 1.18 (d, J=6 Hz), 1.24-1.29 (m), 1.33-1.47 (m), 1.54 (dd, J=4.8 Hz, 15.2 Hz), 1.66 (m), 1.80-2.01 (m), 2.06-2.12 (m), 2.18-2.24 (m), 2.26 (s), 2.28-2.31 (m), 2.35-2.41 (m), 2.51 (d, J=10 Hz), 2.65-2.96 (m), 3.12 (s), 3.22-3.29 (m), 3.41-3.47 (m), 3.54-3.69 (m), 3.81 (d, J=13.2 Hz), 4.11 (m), 4.24 (m), 4.45 (t, J=7.0 Hz), 4.50 (d, J=6.8 Hz), 4.75 (d, J=7.2 Hz), 4.97 (d, J=5.2 Hz), 7.42 (d, J=8.0 Hz), 7.84 (d, J=8.0 Hz), 8.35 (s). MS (FAB, m/ba) 1021.2 (M+H)<sup>+</sup>.

Synthesis of Descladinose-Azithromycin-N-phenyl-  
triazolylhexa-hydroxamic acid (8)

Method A

To a solution of compound 18 (0.04 g, 0.05 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H<sub>2</sub>O) (0.04 mL, 0.54 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and saturated sodium bicarbonate (25 mL), the two layers were separated and the aqueous layer was extracted with 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (2×20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH 10:1:0.1 to give compound 8 (9.0 mg, 23%) as brown-white solid.

Method B

Reaction of descladinose-azithromycin-N-phenylacetylene 4 (0.134 g, 0.188 mmol) and 6-azido-hexahydroxamic acid 15a (0.130 g, 0.755 mmol) within 8 h (according to the protocols of Method B described for the synthesis of compound 7 above), followed by prep TLC (silica, 10:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) gave 73 mg (43%) of 8 as a brownish white solid.

<sup>1</sup>H-NMR (Acetone-d<sub>6</sub>, 400 MHz) δ 0.82-0.90 (m), 1.02 (d, J=7.2 Hz), 1.07 (s), 1.09 (d, J=6.8 Hz), 1.18-1.23 (m), 1.28 (bs), 1.31-1.39 (m), 1.46-1.56 (m), 1.65 (m), 1.81-1.83 (m),



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1.87-1.99 (m), 2.05-2.11 (m), 2.18-2.21 (m), 2.18-2.21 (m), 2.24 (s), 2.25-2.29 (m), 2.35 (s), 4.47 (d, J=9.2 Hz), 2.61-2.67 (m), 2.70-2.77 (m), 3.30-3.34 (m), 3.41 (m), 3.52-3.65 (m), 2.81 (d, J=13.2 Hz), 4.44 (t, J=7.0 Hz), 4.59 (d, J=7.6 Hz), 4.87 (dd, J=1.8 Hz, 11.0 Hz), 7.43 (d, J=8.4 Hz), 7.83 (d, J=8.4 Hz), 8.34 (m); HMRS (ESI) calcd for  $[C_{44}H_{74}N_6O_{11}+H]^+$  863.5488. found 863.5528.

Accordingly, compounds 9-14 and 23-30 (in this section) were synthesized according to the protocols of Method B described for the synthesis of compound 7 above.

Synthesis of  
Azithromycin-N-phenyltriazolyheptahydroxamic  
acid (9)

Reaction of azithromycin-N-phenylacetylene 3 (0.134 g, 0.158 mmol) and 7-azidoheptahydroxamic acid 15b (0.125 g, 0.672 mmol) within 4 h, followed by prep TLC (silica, 12:1:0.1  $CH_2Cl_2$ /MeOH/conc.  $NH_4OH$ ) gave 93 mg (56%) of 9 as a brownish white solid.  $^1H$ -NMR ( $CDCl_3$ , 400 MHz)  $\delta$  0.81-1.51 (m), 1.54-1.65 (m), 1.70-2.14 (m), 2.20-2.38 (m), 2.46-2.56 (m), 2.60-2.70 (m), 3.00 (s), 3.31 (t, J=8.8 Hz), 3.38-3.54 (m), 3.60 (s), 3.78 (d, J=12.8 Hz), 3.98-4.20 (m), 4.36 (d, J=7.2 Hz), 4.49 (d, J=7.2 Hz), 5.11 (d, J=4.0 Hz), 7.32 (d, J=7.6 Hz), 7.73 (s), 7.75 (d, J=7.6 Hz);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$  6.6, 8.8, 11.5, 14.4, 16.6, 17.7, 21.3, 21.6, 21.8, 25.1, 26.0, 26.7, 27.1, 28.2, 29.2, 29.6, 30.0, 33.1, 34.5, 35.7, 36.7, 41.8, 42.7, 45.3, 49.3, 50.3, 50.7, 57.9, 62.7, 63.0, 65.8, 68.6, 69.4, 70.4, 72.6, 73.2, 73.8, 77.8, 78.1, 78.2, 83.5, 94.4, 102.8, 119.3, 125.7, 129.4, 129.7, 138.4, 147.4, 171.3, 178.4; HMRS (ESI) calcd for  $[C_{53}H_{90}N_6O_{14}+H]^+$  1035.6587. found 1035.6628.

Synthesis of Descladinose-Azithromycin-N-phenyl-  
triazolyheptahydroxamic acid (10)

Reaction of descladinose-azithromycin-N-phenylacetylene 4 (0.130 g, 0.188 mmol) and 7-azidoheptahydroxamic acid 15b (0.130 g, 0.755 mmol) within 8 h, followed by prep TLC (silica, 10:1:0.1  $CH_2Cl_2$ /MeOH/conc.  $NH_4OH$ ) gave 78 mg (47%) of 10 as a brownish white solid.  $^1H$ -NMR ( $CDCl_3$ , 400 MHz)  $\delta$  0.66-2.32 (m), 2.47 (d, J=10.8 Hz), 2.63-2.70 (m), 3.34-3.51 (m), 3.62-3.69 (m), 4.20-4.40 (m), 4.74 (br s), 7.26 (br s), 7.73 (br s);  $^{13}C$  NMR ( $CDCl_3$ , 100 MHz)  $\delta$  7.4, 7.9, 10.7, 16.0, 16.1, 20.9, 21.1, 25.0, 25.7, 26.5, 28.0, 28.9, 29.8, 35.8, 36.3, 36.9, 42.0, 44.4, 50.1, 57.9, 62.7, 63.9, 69.9, 70.4, 70.8, 73.3, 74.1, 75.2, 79.5, 94.9, 106.6, 119.7, 125.7, 129.2, 129.6, 138.4, 147.3, 177.5; HMRS (EST) calcd for  $[C_{45}H_{76}N_6O_{11}+H]^+$  877.5645. found 877.5665.

Synthesis of  
Azithromycin-N-phenyltriazolyloctahydroxamic acid  
(11)

Reaction of azithromycin-N-phenylacetylene 3 (0.10 g, 0.120 mmol) and 8-azido-octahydroxamic acid 15c (0.047 g, 0.24 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1  $CH_2Cl_2$ /MeOH/conc.  $NH_4OH$ ) gave 72 mg (58%) of 11 as a brownish white solid.  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  0.85 (t, J=4.0 Hz), 0.87-1.22 (m), 1.29 (s), 1.30-2.28 (m), 2.29 (s), 2.30-3.00 (m), 3.10 (s), 3.20-3.79 (m), 3.99-4.03 (m), 4.35-4.40 (m), 4.65 (d, J=8.0 Hz), 5.11 (d, J=4.8 Hz), 7.34 (d, J=8.0 Hz), 7.72 (s), 7.77 (d, J=8.0 Hz);  $^{13}C$  NMR ( $DMSO-d_6$ , 400 MHz)  $\delta$  7.5, 9.8, 11.6, 15.4, 18.2, 19.1, 21.5, 22.1, 22.7, 25.6, 26.3, 26.6, 28.7, 29.0, 29.6, 30.2, 32.0, 32.8, 35.2, 36.4, 37.2, 42.2, 45.3, 49.2, 50.1, 58.3, 63.2, 65.4, 67.7, 70.8, 73.3, 74.2, 77.0, 78.4, 83.4, 102.8, 121.6, 125.6, 129.7, 130.0, 135.0,

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147.0, 177.8; HRMS (FAB, thioglycerol) calc for  $[C_{54}H_{92}N_6O_{14}+H]^+$  1049.6749. found 1049.6648.

Synthesis of Descladinose-Azithromycin-N-phenyl-  
triazolyloctahydroxamic acid (12)

Reaction of azithromycin-N-phenylacetylene 4 (0.10 g, 0.144 mmol) and 8-azido-octahydroxamic acid 15c (0.049 g, 0.246 mmol) within 2.5 h, followed by prep TLC (silica, 10:1:0.1  $CH_2Cl_2$ /MeOH/conc.  $NH_4OH$ ) gave 94 mg (73%) of 12 as a brownish white solid. HRMS (FAB, thioglycerol) calc for  $[C_{46}H_{79}N_6O_{11}+H]^+$  891.5806. found 891.5910.

Synthesis of  
Azithromycin-N-phenyltriazolylnonahydroxamic  
acid (13)

Reaction of azithromycin-N-phenylacetylene 3 (0.10 g, 0.120 mmol) and 9-azido-nonahydroxamic acid 15d (0.043 g, 0.20 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1  $CH_2Cl_2$ /MeOH/conc.  $NH_4OH$ ) gave 64 mg (51%) of 13 as a brownish white solid.  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  0.84-1.30 (m), 1.33-2.26 (m), 2.30 (s), 2.38-2.68 (m), 2.99 (s), 3.32-3.84 (m), 4.03-4.08 (m), 4.35-4.41 (m), 4.53 (d, J=8.0 Hz), 5.13 (d, J=4.0 Hz), 7.35 (d, J=8.0 Hz), 7.75 (s), 7.78 (d, J=8.0 Hz);  $^{13}C$  NMR ( $DMSO-d_6$ , 400 MHz)  $\delta$  6.9, 9.0, 11.6, 14.7, 16.9, 18.0, 21.6, 21.8, 22.1, 25.6, 26.4, 26.9, 27.3, 28.8, 29.0, 29.1, 29.5, 29.9, 30.4, 34.8, 36.0, 37.0, 42.1, 43.0, 45.6, 49.5, 50.5, 58.1, 63.5, 66.1, 68.8, 70.7, 72.9, 74.1, 78.1, 78.3, 78.5, 83.7, 94.4, 94.7, 103.1, 119.6, 126.0, 129.7, 130.0, 147.6, 178.7; LRMS (MALDI) calc for  $[C_{55}H_{94}N_6O_{14}+H]^+$  1063.6. found 1063.7.

Synthesis of  
Azithromycin-N-phenyltriazolyldecahydroxamic  
acid (14)

Reaction of azithromycin-N-phenylacetylene 3 (0.10 g, 0.120 mmol) and 10-azido-decahydroxamic acid 15e (0.045 g, 0.20 mmol) within 4.5 h, followed by prep TLC (silica, 12:1:0.1  $CH_2Cl_2$ /MeOH/conc.  $NH_4OH$ ) gave 70 mg (56%) of 14 as a brownish white solid.  $^1H$  NMR ( $CDCl_3$ , 400 MHz)  $\delta$  0.85-1.36 (m), 1.41-2.24 (m), 2.28, 2.36 (s), 2.33-3.10 (m), 3.05 (s), 3.23-3.82 (m), 4.06-4.10 (m), 4.36-4.41 (m), 4.49 (d, J=8.0 Hz), 5.15 (d, J=4.0 Hz), 7.34 (d, J=8 Hz), 7.75 (s), 7.78 (d, J=8.0 Hz);  $^{13}C$  NMR ( $DMSO-d_6$ , 400 MHz)  $\delta$  6.7, 9.0, 11.7, 14.6, 16.9, 17.9, 21.6, 21.9, 22.0, 25.6, 26.4, 26.8, 27.0, 27.3, 28.8, 29.0, 21.9, 29.3, 29.5, 29.9, 30.3, 33.6, 34.9, 35.8, 37.0, 42.1, 43.0, 45.6, 49.6, 50.6, 51.6, 58.0, 62.8, 63.9, 66.2, 68.9, 69.6, 70.7, 72.9, 73.5, 74.0, 74.1, 78.2, 78.6, 83.6, 94.6, 103.0, 119.6, 126.0, 129.6, 129.9, 138.9, 147.6, 178.6; HRMS (MALDI) calc for  $[C_{56}H_{96}N_6O_{14}+H]^+$  1077.7057. found 1077.6971.

Synthesis of Clarithromycin-N-phenylacetylene (20)

To a solution of N-demethylated clarithromycin 19 (2.40 g, 3.34 mmol) in anhydrous DMSO (30 mL) was added Hunig's base (3 mL) and 4-ethynylbenzyl methanesulfonate 2 (0.920 g, 4.34 mmol). The reaction mixture was then heated with stirring under argon at 85° C. for 2.5 h. The reaction was cooled and diluted with EtOAc (100 mL) and washed with saturated  $NaHCO_3$  (3x60 mL) and saturated brine (60 mL). The organic layer was dried over  $Na_2SO_4$  and concentrated in vacuo. The crude product was purified by flash chromatography (silica, gradient 12:1; 10:1; 8:1;  $CH_2Cl_2$ /acetone) to give 1.8 g (63%) of 20 as a brownish white solid.  $^1H$ -NMR

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(CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.82 (t, J=7.2 Hz), 1.03-1.28 (m), 1.37 (s), 1.40-1.55 (m), 1.65-1.90 (m), 2.03 (d, J=10.0 Hz), 2.22 (s), 2.30 (d, J=15.2 Hz), 2.40-2.60 (m), 2.80-2.90 (m), 2.94-3.00 (m), 3.04 (s), 3.09 (s), 3.16 (s), 3.24-3.29 (m), 3.38-3.46 (m), 3.59 (d, J=6.8 Hz), 3.70-3.75 (m), 3.88-3.95 (m), 4.37 (d, J=7.2 Hz), 4.88 (d, J=4.4 Hz), 5.02 (dd, J=11.6, 2.4 Hz), 7.23 (d, J=12.0 Hz), 7.42 (d, J=8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  9.2, 10.7, 12.4, 16.1, 18.1, 18.7, 19.9, 21.1, 21.5, 29.3, 32.4, 34.8, 36.9, 37.2, 39.2, 45.0, 45.2, 49.3, 50.6, 53.4, 57.6, 63.3, 65.6, 68.5, 69.0, 70.6, 72.5, 74.2, 76.5, 77.8, 78.1, 78.2, 80.8, 95.8, 102.5, 120.9, 128.6, 132.0, 133.5, 139.4, 175.4; HRMS (ESI) calc for [C<sub>46</sub>H<sub>73</sub>NO<sub>13</sub>+H]<sup>+</sup> 848.5155. found 848.5181.

Synthesis of  
Descladinose-Clarithromycin-N-phenylacetylene  
(21)

To a solution of clarithromycin-N-phenylacetylene 20 (0.500 g, mmol) in ethanol (20 mL) was added 1N HCl (20 mL), and stirring continued for 22 h at room temperature. The reaction mixture was basified with concentrated NH<sub>4</sub>OH to about pH=9. The reaction mixture was diluted with distilled water (40 mL) and extracted with EtOAc (3×60 mL). The combined organic layers were washed with saturated brine (40 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo. The crude product was purified by flash chromatography (silica, 8:1 CH<sub>2</sub>Cl<sub>2</sub>/acetone) to give 320 mg (79%) of 21 as a brownish white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.82 (t, J=7.6 Hz), 1.09-1.28 (m), 1.34 (s), 1.40-1.55 (m), 1.70-1.74 (m), 1.87-1.94 (m), 2.08-2.15 (m), 2.54-2.66 (m), 2.94-2.98 (m), 3.05 (s), 3.25 (s), 3.31-3.42 (m), 3.48-3.56 (m), 3.66 (d, J=10.0 Hz), 3.82 (s), 3.90 (s), 4.35 (d, J=7.6 Hz), 5.14 (dd, J=10.8, 2.0 Hz), 7.18 (d, J=8.0 Hz), 7.42 (d, J=8.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  8.4, 10.5, 12.7, 15.3, 16.3, 17.8, 18.8, 21.4, 29.2, 35.9, 36.6, 37.5, 38.7, 44.5, 45.5, 49.6, 57.8, 65.0, 69.7, 70.1, 70.6, 74.1, 77.9, 78.9, 83.3, 88.5, 106.5, 121.0, 128.4, 132.1, 139.1, 174.7; HRMS (ESI) calc for [C<sub>38</sub>H<sub>59</sub>NO<sub>10</sub>+H]<sup>+</sup> 690.4212. found 690.4259.

Synthesis of  
Clarithromycin-N-phenyltriazylohexahydroxamic  
acid (23)

Reaction of clarithromycin-N-phenylacetylene 20 (0.100 g, 0.120 mmol) and 6-azidohexahydroxamic acid 15a (0.080 g, 0.470 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) gave 70 mg (58%) of 23 as a brownish white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.81 (t, J=7.6 Hz), 1.03-1.52 (m), 1.62-1.92 (m), 2.04-2.29 (m), 2.48-2.60 (m), 2.82-2.90 (m), 2.93-2.99 (m), 3.09 (s), 3.19 (s), 3.28-3.33 (m), 3.42-3.46 (m), 3.60 (d, J=7.6 Hz), 3.70-3.80 (m), 3.90-3.98 (m), 4.37-4.40 (m), 4.87 (d, J=4.8 Hz), 5.03 (dd, J=11.6, 2.4 Hz), 7.34 (d, J=7.6 Hz), 7.77 (d, J=7.6 Hz), 7.82 (s); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  9.1, 10.5, 12.2, 15.9, 17.9, 18.5, 19.7, 20.9, 21.2, 21.4, 24.3, 25.5, 29.4, 29.6, 34.7, 36.8, 37.1, 39.0, 39.1, 45.0, 45.1, 49.3, 49.9, 50.5, 53.3, 57.5, 63.6, 65.5, 68.5, 69.0, 70.7, 72.4, 74.2, 77.8, 78.2, 80.9, 95.9, 102.6, 119.8, 125.6, 129.4, 147.4, 175.8; HMRS (ESI) calcd for [C<sub>52</sub>H<sub>85</sub>N<sub>5</sub>O<sub>15</sub>+H]<sup>+</sup> 1020.6114. found 1020.6121.

Synthesis of Descladinose-Clarithromycin-N-phenyltriazylohexahydroxamic acid (24)

Reaction of descladinose-clarithromycin-N-phenylacetylene 21 (0.075 g, 0.109 mmol) and 6-azidohexahydroxamic

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acid 15a (0.040 g, 0.233 mmol) within 4 h, followed by prep TLC (silica, 10:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) gave 47 mg (51%) of 24 as a brownish white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.79 (t, J=7.2 Hz), 1.08-1.32 (m), 1.39-1.64 (m), 1.71-1.81 (m), 1.82-1.96 (m), 2.04-2.18 (m), 2.51-2.70 (m), 2.92-2.98 (m), 3.18-3.38 (m), 3.45-3.55 (m), 3.60-3.74 (m), 3.81 (s), 3.90 (s), 4.33 (br s), 5.13 (d, J=10.4 Hz), 7.29 (br s), 7.74 (br s); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  8.6, 10.7, 12.9, 15.5, 16.4, 18.0, 19.0, 21.5, 21.6, 29.5, 29.9, 36.1, 36.7, 37.7, 39.0, 44.7, 45.7, 49.8, 50.3, 58.2, 64.6, 70.0, 70.3, 70.9, 74.4, 78.3, 79.1, 88.5, 106.7, 120.3, 126.1, 129.6, 129.9, 147.7, 175.4; HMRS (ESI) calcd for [C<sub>44</sub>H<sub>71</sub>N<sub>5</sub>O<sub>12</sub>+H]<sup>+</sup> 862.5172. found 862.5155.

Synthesis of  
Clarithromycin-N-phenyltriazyloheptahydroxamic  
acid (25)

Reaction of clarithromycin-N-phenylacetylene 20 (0.130 g, 0.153 mmol) and 7-azidoheptahydroxamic acid 15b (0.105 g, 0.565 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) gave 105 mg (67%) of 25 as yellowish solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.82 (t, J=8.0 Hz), 1.04-1.52 (m), 1.67-1.92 (m), 2.14-2.29 (m), 2.52-2.60 (m), 2.82-2.90 (m), 2.95-3.00 (m), 3.10 (s), 3.16 (s), 3.27-3.32 (m), 3.41-3.46 (m), 3.59 (d, J=6.8 Hz), 3.69-3.79 (m), 3.90-3.95 (m), 4.34-4.39 (m), 4.87 (d, J=4.4 Hz), 5.02 (d, J=9.2 Hz), 7.33 (d, J=6.4 Hz), 7.77 (d, J=8.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  9.1, 10.5, 12.2, 15.9, 17.9, 18.5, 19.8, 20.9, 21.2, 21.4, 24.7, 25.5, 27.7, 29.8, 34.7, 36.8, 37.1, 39.1, 45.0, 45.2, 49.3, 50.0, 50.5, 57.6, 63.6, 65.6, 68.6, 69.0, 70.7, 72.4, 74.2, 77.8, 78.3, 80.9, 95.9, 102.7, 119.5, 125.7, 129.4, 147.5, 175.8; HMRS (ESI) calcd for [C<sub>53</sub>H<sub>87</sub>N<sub>5</sub>O<sub>15</sub>+H]<sup>+</sup> 1034.6271. found 1034.6246.

Synthesis of Descladinose-Clarithromycin-N-phenyltriazyloheptahydroxamic acid (26)

Reaction of descladinose-clarithromycin-N-phenylacetylene 21 (0.075 g, 0.109 mmol) and 7-azidoheptahydroxamic acid 15b (0.040 g, 0.233 mmol) within 4 h, followed by prep TLC (silica, 10:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) gave 80 mg (84%) of 26 as a brownish white solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.78 (t, J=7.2 Hz), 1.06-1.31 (m), 1.40-1.53 (m), 1.71 (d, J=11.6 Hz), 1.80-1.91 (m), 2.01-2.20 (m), 2.50-2.65 (m), 2.91-2.97 (m), 3.16 (t, J=6.4 Hz), 3.26-3.35 (m), 3.42-3.54 (m), 3.64-3.71 (m), 3.80 (br s), 3.90 (br s), 4.30-4.34 (m), 5.12 (dd, J=11.6, 2.4 Hz), 7.27 (d, J=8.0 Hz), 7.72 (d, J=7.2 Hz), 7.80 (s); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  8.3, 10.3, 12.5, 15.2, 16.1, 17.6, 18.6, 21.1, 21.3, 24.8, 25.1, 25.5, 26.2, 27.8, 28.5, 29.1, 29.6, 29.7, 32.3, 35.8, 36.3, 37.4, 38.6, 44.3, 45.4, 49.5, 50.0, 51.2, 57.8, 64.2, 69.7, 69.9, 70.6, 74.1, 77.9, 78.7, 88.0, 106.3, 119.9, 125.7, 129.3, 129.5, 138.3, 147.3, 175.1; HMRS (ESI) calcd for [C<sub>45</sub>H<sub>73</sub>N<sub>5</sub>O<sub>12</sub>+H]<sup>+</sup> 876.5329. found 876.5301.

Synthesis of  
Clarithromycin-N-Phenyltriazyloctahydroxamic  
acid (27)

Reaction of clarithromycin-N-phenylacetylene 20 (0.101 g, 0.120 mmol) and 8-azidoctahydroxamic acid 15c (0.047 g, 0.24 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) gave 92 mg (74%) of 27 as a brownish white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  0.81 (t, J=7.2 Hz), 1.04-2.05 (m), 2.22 (s), 2.19-2.82 (m), 3.00 (s), 3.08 (s), 2.91-3.80 (m), 3.95 (m), 4.38 (m), 4.88 (d,

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J=4.4 Hz), 5.04 (dd, J=10.8, 2.0 Hz), 7.33 (d, J=7.6 Hz), 7.71 (s), 7.77 (d, J=8.0 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.8, 9.4, 10.8, 12.5, 16.2, 18.2, 18.8, 20.0, 21.2, 21.5, 21.6, 25.1, 26.0, 26.7, 28.2, 28.6, 28.9, 29.9, 30.1, 35.0, 36.9, 37.4, 39.2, 39.4, 45.2, 45.4, 46.1, 49.6, 50.4, 50.8, 51.6, 58.1, 63.6, 65.9, 68.4, 69.3, 70.9, 72.8, 74.4, 78.0, 78.5, 81.2, 96.1, 120.1, 102.6, 126.1, 130.2, 147.4, 176.0; HRMS (ESI) calc for  $[\text{C}_{54}\text{H}_{90}\text{N}_5\text{O}_{15}+\text{H}]^+$  1048.6427. found 1048.6486.

Synthesis of Descladinose-Clarithromycin-N-Phenyltriazyloctahydroxamic acid (28)

Reaction of clarithromycin-N-phenylacetylene 21 (0.10 g, 0.144 mmol) and 8-azidoctahydroxamic acid 15c (0.049 g, 0.246 mmol) within 2.5 h, followed by prep TLC (silica, 10:1:0.1  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{conc. NH}_4\text{OH}$ ) gave 117 mg (90%) of 28 as a brownish white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.81 (t, J=7.2 Hz), 1.10-2.09 (m), 2.18 (s), 2.19-2.68 (m), 2.98-3.73 (m), 3.83 (s), 3.93 (m), 4.36 (m), 5.16 (d, J=8.0 Hz), 7.31 (d, J=8.0 Hz), 7.77 (2H, d, J=8.0 Hz), 7.79 (1H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  8.5, 10.6, 12.8, 15.4, 16.4, 17.9, 18.9, 21.4, 21.6, 29.4, 36.1, 36.7, 37.7, 38.9, 44.7, 45.7, 49.8, 58.0, 65.2, 70.0, 70.4, 70.8, 74.4, 76.8, 78.2, 79.2, 83.6, 88.8, 106.9, 121.3, 128.7, 132.5, 139.6, 175.2; HRMS (FAB, thioglycerol) calc for  $[\text{C}_{46}\text{H}_{76}\text{N}_5\text{O}_{12}+\text{H}]^+$  890.5490. found 890.5562.

Synthesis of  
Clarithromycin-N-Phenyltriazylnonahydroxamic acid (29)

Reaction of clarithromycin-N-phenylacetylene 20 (0.100 g, 0.120 mmol) and 9-azidononahydroxamic acid 15d (0.043 g, 0.20 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{conc. NH}_4\text{OH}$ ) gave 54 mg (42%) of 29 as a brownish white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.81 (t, J=7.2 Hz), 1.04-2.02 (m), 2.24 (s), 2.10-2.97 (m), 3.00 (s), 3.09 (s), 3.20-3.82 (m), 3.88 (m), 4.39 (m), 4.88 (d, J=4.0 Hz), 5.05 (d, J=10.0 Hz), 7.35 (d, J=8.0 Hz), 7.77 (d, J=4.0 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.0, 9.3, 10.8, 12.5, 16.1, 18.2, 18.8, 21.2, 21.5, 21.6, 21.7, 21.8, 26.3, 26.8, 28.7, 28.9, 29.1, 29.9, 30.3, 35.0, 37.0, 37.4, 39.3, 39.4, 45.2, 45.4, 49.6, 50.5, 50.8, 51.6, 57.8, 63.8, 65.8, 68.8, 69.2, 70.9, 72.7, 74.5, 76.8, 78.1, 78.4, 78.5, 81.1, 96.1, 102.9, 119.7, 125.9, 129.6, 129.9, 138.7, 147.6, 176.1; FIRMS (ESI) calc for  $[\text{C}_{55}\text{H}_{91}\text{N}_5\text{O}_{15}+\text{H}]^+$  1062.6584. found 1062.6586.

Synthesis of  
Clarithromycin-N-Phenyltriazyldecahydroxamic acid (30)

Reaction of clarithromycin-N-phenylacetylene 20 (0.10 g, 0.120 mmol) and 10-azidodecahydroxamic acid 15e (0.045 g, 0.197 mmol) within 2.5 h, followed by prep TLC (silica, 12:1:0.1  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{conc. NH}_4\text{OH}$ ) gave 68 mg (53%) of 30 as a brownish white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.82 (t, J=7.2 Hz), 1.05-2.12 (m), 2.24 (s), 2.26-2.97 (m), 3.01, 3.10 (s), 3.19-3.80 (m), 3.95 (m), 4.39 (m), 4.89 (d, J=4.0 Hz), 5.04 (d, J=8.0 Hz), 7.35 (d, J=8.0 Hz), 7.76 (s), 7.79 (d, J=8.0 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.3, 10.8, 12.5, 16.1, 18.2, 18.8, 20.0, 21.2, 21.5, 21.7, 25.4, 26.2, 28.9, 29.0, 29.3, 29.6, 29.9, 30.2, 35.0, 37.0, 37.4, 39.3, 39.4, 45.2, 45.4, 49.6, 50.5, 50.8, 51.6, 57.8, 63.8, 65.8, 68.8, 69.2, 70.9, 72.7, 74.5, 76.8, 78.1, 78.5, 81.1, 96.1, 102.9, 119.7, 125.9, 129.6, 129.8, 138.9, 147.6, 176.1; HRMS (ESI) calc for  $[\text{C}_{56}\text{H}_{93}\text{N}_5\text{O}_{15}+\text{H}]^+$  1076.6740. found 1076.6667.

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## Example 2

## Synthesis of Compounds 36 and 38 in Table 3

Synthesis of Methyl  
8-(4-(hydroxymethyl)phenylamino)-8-oxooctanoate  
(33)

To a mixture of 8-methoxy-8-oxooctanoic acid (0.40 g, 2.10 mmol), benzotriazole (0.28 g, 2.23 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (15 mL) was added  $\text{SOCl}_2$  (0.17 mL, 2.23 mmol) at  $0^\circ\text{C}$ , the mixture was kept stirring at  $0^\circ\text{C}$  for 2.5 h and then filtered. The solvent was evaporated off to give crude acid chloride 31 which was used without further purification.

To a solution of (4-aminophenyl)methanol 32 (0.31 g, 2.50 mmol) in anhydrous pyridine (8 mL) was added chlorotrimethylsilane (0.32 mL, 2.50 mmol) at room temperature and stirring continued for 2 h. The mixture, together with a catalytic amount of DMAP, was added to a mixture of crude chloride 31 (obtained as described above) in pyridine at  $0^\circ\text{C}$ . The reaction was allowed to warm to room temperature and stirring continued overnight. Water (5 mL) and 1 M TBAF in tetrahydrofuran (THF) (0.25 mL, 0.25 mmol) were added and stirring continued for additional 30 min. EtOAc (50 mL) and 1N HCl (30 mL) were added, the two layers were separated, the organic layer was washed with 1N HCl (30 mL) and saturated brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . Solvent was evaporated off and the crude was purified by preparative TLC, eluting with acetone/hexanes 1:1 to give compound 33 (195 mg, 30%) as yellow-white solid.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  1.24 (4H, m), 1.49-1.59 (4H, m), 2.17-2.25 (4H, m), 3.58 (3H, s), 4.50 (2H, s), 7.13 (2H, d, J=8.4 Hz), 7.37 (2H, d, J=8.4 Hz).

Synthesis of Azithromycin-arylalkyl methyl ester  
(35)

To a solution of crude preparation of compound 33 (0.64 g, 2.20 mmol) in  $\text{CH}_2\text{Cl}_2$  (15 mL) and triethylamine ( $\text{Et}_3\text{N}$ ) (0.90 mL, 6.60 mmol) was added mesyl chloride (0.70 mL, 8.85 mmol) at  $0^\circ\text{C}$  and the reaction was allowed to warm to room temperature. Stirring continued for 2 h,  $\text{CH}_2\text{Cl}_2$  (40 mL) and saturated sodium bicarbonate (30 mL) were added. The two layers were separated; the organic layer was washed with sodium bicarbonate (1x30 mL), saturated brine (30 mL) and dried over  $\text{Na}_2\text{SO}_4$ . Solvent was evaporated off and the crude was purified by flash chromatography (silica gel, eluting with Hexane/EtOAc, gradient 3:1, 2:1, 1:1) to give compound 34 (320 mg, 40%) as white solid.

A mixture of 4'-Desmethylazithromycin 1 (0.45 g, 0.62 mmol), compound 34 (0.32 g, 0.86 mmol), catalytic amount of potassium iodide in THF (15 mL) and Hunig's base (3 mL) was heated under refluxing condition for 48 h.  $\text{CH}_2\text{Cl}_2$  (80 mL) and saturated sodium bicarbonate (40 mL) were added and the two layers were separated. The organic layer was washed with sodium bicarbonate (40 mL), saturated brine (40 mL) and dried over  $\text{Na}_2\text{SO}_4$ . Solvent was evaporated off and the crude was purified by preparative TLC, eluting with EtOAc/hexanes/ $\text{Et}_3\text{N}$  3:2:0.1 to give compound 35 (176 mg, 28%) as brown-white solid.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.79-0.83 (m), 0.94-1.01 (m), 1.09-1.20 (m), 1.22-1.32 (m), 1.37-1.59 (m), 1.62-1.73 (m), 1.77-2.01 (m), 2.08-2.28 (m), 2.38-2.52 (m), 2.61-2.71 (m), 2.91-3.01 (m), 3.11 (s), 3.25-3.33 (m), 3.42 (m), 3.54-3.65 (m), 3.97 (m), 4.18 (m), 4.36 (d, J=6.8 Hz), 4.61 (m), 5.03 (d, J=4.4 Hz), 7.14 (d, J=8.4 Hz), 7.42 (d, J=8.4 Hz), 7.73 (s), 8.97 (bs);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  7.5, 9.1, 11.3, 14.8, 16.2, 16.9, 18.2, 20.5, 21.2, 21.4,

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21.5, 21.9, 24.6, 25.3, 26.7, 27.5, 28.7, 29.6, 33.9, 34.7, 36.2, 36.7, 37.3, 39.1, 41.9, 42.2, 45.1, 48.5, 49.3, 51.4, 57.3, 62.2, 64.3, 65.4, 68.5, 69.9, 70.5, 72.7, 73.5, 73.8, 74.1, 77.7, 77.9, 83.4, 94.4, 102.6, 119.5, 129.0, 134.2, 137.0, 171.0, 173.8, 178.4. MS (FAB, mmba) 1010.3 (M+H)<sup>+</sup>.

#### Synthesis of Azithromycin-arylalkyl hydroxamic acid (36)

To a solution of compound 35 (0.09 g, 0.09 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H<sub>2</sub>O) (0.03 mL, 0.54 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and saturated sodium bicarbonate (25 mL), the two layers were separated and the aqueous layer was extracted with 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (2×20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH 10:1:0.1 to give compound 36 (22 mg, 25%) as brown-white solid. <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz) δ 0.87-0.92 (m), 1.02-1.12 (m), 1.17-1.37 (m), 1.43-1.69 (m), 1.75-1.88 (m), 1.99 (m), 2.08 (m), 2.13-2.19 (m), 2.24 (s), 2.30 (s), 2.33-2.41 (m), 2.54 (d, J=11.2 Hz), 2.75-2.80 (m), 3.00 (d, J=9.6 Hz), 3.19 (bs), 3.47-3.51 (m), 3.60 (bs), 3.63-3.78 (m), 4.14-4.22 (m), 4.50 (d, J=7.2 Hz), 5.02 (d, J=4.8 Hz), 7.29 (d, J=8.0 Hz), 7.49 (d, J=8.4 Hz). MS (FAB, mmba) 1011.3 (M+H)<sup>+</sup>.

#### Synthesis of Desclasinose-azithromycin-arylalkyl hydroxamic acid (38)

A mixture of compound 35 (0.05 g, 0.05 mmol) in 0.25 N HCl (15 mL) was stirred at room temperature for 20 h and poured into EtOAc (20 mL). The two layers were separated and the aqueous layer was washed with EtOAc (2×20 mL), basified with concentrated NH<sub>4</sub>OH and then extracted with 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (2×30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off to give compound 37 which was used for the next reaction without further purification.

To a solution of compound 37 (obtained as described above) in 1:1 THF/MeOH (2 mL) was added hydroxylamine (50% in H<sub>2</sub>O) (0.05 mL, 0.79 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The reaction was partitioned between 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and saturated brine (20 mL), the two layers were separated and the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH/NH<sub>4</sub>OH 9:1:0.1 to give compound 38 (7 mg, 16%) as brown-white solid. <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz) δ 0.78 (m), 0.85 (d, J=7.2 Hz), 0.91 (d, J=8.0 Hz), 0.99 (s), 1.11 (m), 1.26-1.76 (m), 1.98 (t, J=7.4 Hz), 2.08 (m), 2.17 (s), 2.25 (t, J=7.4 Hz), 2.40 (bs), 2.58 (m), 2.95 (bs), 3.24 (m), 3.37-3.56 (m), 3.67 (d, J=13.2 Hz), 4.53 (d, J=7.6 Hz), 7.19 (d, J=8.4 Hz), 7.39 (d, J=8.4 Hz). MS (FAB, mmba) 853.3 (M+H)<sup>+</sup>.

#### Example 3

#### Synthesis of Compounds 40, 44 and 47 in Table 3

#### Synthesis of Azithromycin-N-phenyltriazolylhepta-2-methyl ketone (40)

Compound 3 (0.040 g, 0.047 mmol) and azido-2-methyl ketone 39 (0.011 g, 0.071 mmol) were dissolved in anhydrous

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THF (7 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.053 mmol) and Hunigs' base (0.1 mL) were then added to reaction mixture and stirring continued for 2 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (3×30 mL) and saturated NH<sub>4</sub>Cl (30 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by preparative TLC (12:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH) to give 38 mg (81%) of 40 as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.86-0.89 (t, J=7.2 Hz), 0.92-0.98 (m), 1.01-1.02 (d, J=7.2 Hz), 1.086 (s), 1.12-1.24 (m), 1.29-1.34 (m), 1.42-1.66 (m), 1.74-1.78 (d, J=16 Hz), 1.84-2.08 (m), 2.11 (s), 2.15 (m), 2.26-2.48 (m), 2.56-2.74 (m), 2.84 (s), 2.74-3.08 (m), 3.11 (s), 3.32-3.40 (m), 3.46-3.84 (m), 3.98-4.46 (m), 4.23 (broad singlet), 4.38-4.51 (m), 4.72 (broad singlet), 5.06-5.12 (m), 7.31-7.38 (m), 7.75 (s), 7.77-7.79 (d, J=8 Hz); HRMS (ESI) calc for [C<sub>53</sub>H<sub>89</sub>N<sub>5</sub>O<sub>13</sub>+H]<sup>+</sup> 1004.6529. found 1004.6482.

#### Synthesis of Azidobenzamide (43)

A solution of azido acid 41 (0.150 g, 0.877 mmol) in dry THF (10 mL) was treated with 1,2-diaminobenzene 42 (0.568 g, 5.26 mmol) and EDC (0.219 g, 1.14 mmol). The resulting mixture was stirred at room temperature for about 24 h and then concentrated in vacuo. The crude was diluted with EtOAc (40 mL), washed in succession with water (30 mL) and brine (30 mL) and the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and the crude was purified by flash chromatography (silica, Hexanes/EtOAc 1:2) to give 79 mg (35%) of 43 as a yellow solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.34-1.68 (m), 2.27-2.31 (m), 3.84 (s), 6.72-6.75 (m), 7.00-7.05 (m), 7.09-7.11 (d, J=8 Hz); HRMS (ESI) calcd for [C<sub>13</sub>H<sub>19</sub>N<sub>5</sub>O+H]<sup>+</sup> 262.1662. found 262.1635.

#### Synthesis of Azithromycin-N-phenyltriazolylheptabenzamide (44)

Compound 3 (0.050 g, 0.059 mmol) and azido benzamide 43 (0.023 g, 0.088 mmol) were dissolved in anhydrous THF (10 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.0526 mmol) and Hunigs' base (0.1 mL) were then added to reaction mixture and stirring continued for 2 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL), washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (3×30 mL) and saturated NH<sub>4</sub>Cl (30 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product is purified by prep TLC (12:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH) to give 38 mg (59%) of 44 as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.80-1.57 (m), 1.70-1.75 (m), 1.85-1.91 (m), 2.01-2.07 (m), 2.13 (s), 2.22-2.26 (m), 2.36-2.41 (m), 2.58 (br s), 2.68 (br s), 2.75-3.07 (m), 3.25-3.62 (m), 3.69 (s), 3.81 (br s), 3.98 (br s), 4.17 (s), 4.32-4.48 (m), 4.72 (br s), 5.05 (s), 6.70-6.76 (m), 6.92-7.02 (m), 7.18-7.21 (d, J=12 Hz), 7.32 (br s), 7.71-7.73 (d, J=8 Hz), 7.78 (s), 7.87 (br s); HRMS (ESI) calcd for [C<sub>59</sub>H<sub>95</sub>N<sub>7</sub>O<sub>13</sub>+H]<sup>+</sup> 1110.7060. found 1110.7012.

#### Synthesis of Descladinose-clarithromycin-N-phenylacetylene-O-Acetate (45)

Descladinose-clarithromycin-N-phenylacetylene 21 (3.80 g, 5.5 mmol) was dissolved in acetone (20 mL) followed by addition of acetic anhydride (0.62 g, 6.0 mmol) and stirred at 40° C. for 36 h. The reaction mixture was diluted with EtOAc (100 mL), washed with aqueous NaHCO<sub>3</sub> and brine, and then purified on silica column eluting with 6:1 CH<sub>2</sub>Cl<sub>2</sub>/Acetone to

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obtain 2.8 g (70%) of 45 as a brownish white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.80 (t, J=7.2 Hz), 0.90 (d, J=7.2 Hz), 1.08-1.47 (m), 1.58 (s), 1.63-2.05 (m), 2.08 (s), 2.16 (s), 2.42-2.80 (m), 2.92 (s), 2.94-3.00 (m), 3.03-3.68 (m), 3.79 (s), 3.94 (s), 4.08 (m), 4.54 (d, J=8.0 Hz), 4.80 (m), 5.15 (dd, J=11.6, 2.4 Hz), 7.17 (d, J=8.4 Hz), 7.38 (d, J=8.0 Hz).

Synthesis of clarithromycin-N-phenylacetylene ketolide (46)

Methyl sulfide (0.35 g, 5.7 mmol), was added to a mixture of N-chlorosuccinimide (0.65 g, 4.8 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (3 mL) while maintaining the temperature at -15° C. Compound 45 (2.5 g, 3.4 mmol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added to the reaction mixture, followed by triethylamine (0.39 g, 3.8 mmol). The mixture was stirred at -15° C. for 3.5 h and partitioned between EtOAc (100 mL) and 0.5 N aqueous NaOH (150 mL). The organic layer was separated, washed with brine (70 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and the crude was purified on silica column eluting with 1:4:0.1 EtOAc/Hexane/Et<sub>3</sub>N, increasing solvent polarity to 2:3:0.1, to afford 2.0 g (80%) of 46 as off-white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.80-0.86 (m), 1.09-1.57 (m), 1.58 (s), 1.62-2.02 (m), 2.05 (s), 2.15 (s), 2.44-2.80 (m), 2.92 (s), 2.95-3.00 (m), 3.05-3.82 (m), 4.12 (m), 4.38 (d, J=8.0 Hz), 4.79-4.83 (m), 5.14 (dd, J=11.2, 2.0 Hz), 7.16 (d, J=7.6 Hz), 7.38 (d, J=7.6 Hz).

Synthesis of Ketolide-N-phenyltriazolylheptabenzamide (47)

Ketolide 46 (0.050 g, 0.069 mmol) and azido benzamide 43 (0.027 g, 0.103 mmol) were dissolved in anhydrous THF (10 mL) and stirred under argon at room temperature. Copper (I) iodide (0.010 g, 0.0526 mmol) and Hunig's base (0.1 mL) were then added to reaction mixture and stirring continued for 2 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (3×30 mL) and saturated NH<sub>4</sub>Cl<sub>2</sub> (30 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude was purified by prep TLC (3:2 CH<sub>2</sub>Cl<sub>2</sub>/Acetone) to give 51 mg (75%) of 47 as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.78-0.88 (m), 1.18-1.25 (m), 1.31-1.70 (m), 1.87-2.00 (m), 2.14 (s), 2.28-2.35 (m), 2.42-2.51 (m), 2.62-2.75 (m), 3.12 (s), 3.31-3.35 (t, J=8 Hz), 3.42 (s), 3.47 (br s), 3.62-3.82 (m), 4.32-4.40 (m), 5.05-5.09 (d, J=16 Hz), 5.44 (s), 5.77 (s), 6.64-6.74 (m), 6.95-7.00 (m), 7.10-7.15 (m), 7.18-7.21 (m), 7.27-7.31 (m), 7.60 (s) 7.72-7.74 (d, J<sup>TM</sup> 8 Hz), 7.77 (s).

Example 4

Synthesis of Compounds 56a-e

The procedure for the synthesis of compounds 56a-e as shown in Scheme 16 is described below.

4'-Desmethylclarithromycin (2)

To a solution of clarithromycin 1 (50.0 g, 68.1 mmol) and sodium acetate (50.3 g, 61.3 mmol) in 80% aqueous methanol (350 mL) at 75-80° C. was added iodine (19.0 g, 74.8 mmol) in three batches within 5 min. The reaction was maintained at pH 8-9 by additions of 1M NaOH (2×50 mL, once at 10 min and 45 min of reaction time). Stirring was continued at 80° C. for 4.5 h. A solution of 5% sodium thiosulfate (300 mL) and dichloromethane (250 mL) were added and the two layers were separated. The aqueous layer was extracted with

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CH<sub>2</sub>Cl<sub>2</sub> (150 mL), the combined organic layers were washed with saturated brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated in vacuo to give 44.0 g of 2, which was used without further purification.

4-Ethynylbenzyl methanesulfonate (3)

To a solution of 4-ethynylbenzyl alcohol (5.0 g, 37.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and triethylamine (Et<sub>3</sub>N) (14.4 mL, 104.0 mmol) was added mesyl chloride (8.04 mL, 104.0 mmol) at 0° C. and the reaction was allowed to warm to room temperature. Stirring continued for 2 h under argon during which TLC revealed a quantitative conversion into a higher Rf product. Ice cold water was added into the reaction mixture and extracted with Et<sub>2</sub>O (200 mL). The organic layer was washed with 1N HCL (70 mL), H<sub>2</sub>O (100 mL), aqueous NaHCO<sub>3</sub> (70 mL), and finally H<sub>2</sub>O (100 mL); and then dried over Na<sub>2</sub>SO<sub>4</sub>. Solvent was evaporated off and dried in vacuo to give 7.0 g of compound 3 as brownish oil which was used without further purification.

4'-Ethynylbenzylclarithromycin (4)

To a solution of 4'-desmethylclarithromycin 2 (2.4 g, 3.34 mmol) in anhydrous DMSO (4 mL), was added Hunig's base (3 mL), and 4-ethynylbenzyl methanesulfonate 2 (0.92 g, 4.34 mmol). The reaction mixture was heated at 85° C. for 2.5 h. The reaction was cooled and diluted with EtOAc (30 mL), then washed with saturated NaHCO<sub>3</sub> (3×10 mL) and saturated brine (10 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated. The crude product was purified on silica column using 12:1 CH<sub>2</sub>Cl<sub>2</sub>/C<sub>2</sub>H<sub>5</sub>CO gradually increasing the solvent polarity to 10:1 and 8:1 to afford 1.8 g (63%) of the title compound.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.82 (3H, t, J=7.2 Hz), 1.03-1.28 (18H, m), 1.37 (3H, s), 1.40-1.55 (3H, m), 1.65-1.90 (6H, m), 2.03 (1H, d, J=10.0 Hz), 2.22 (3H, s), 2.30 (1H, d, J=15.2 Hz), 2.40-2.60 (2H, m), 2.80-2.90 (2H, m), 2.94-3.00 (6H, m), 3.04 (1H, s), 3.09 (3H, s), 3.16 (1H, s), 3.24-3.29 (1H, m), 3.38-3.46 (3H, m), 3.59 (1H, d, J=6.8 Hz), 3.70-3.75 (3H, m), 3.88-3.95 (1H, m), 4.37 (1H, d, J=7.2 Hz), 4.88 (1H, d, 4.4 Hz), 5.02 (1H, dd, 11.6, 2.4 Hz), 7.23 (2H, d, J=12.0 Hz), 7.42 (2H, d, J=8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 9.2, 10.7, 12.4, 16.1, 18.1, 18.7, 19.9, 21.1, 21.5, 29.3, 32.4, 34.8, 36.9, 37.2, 39.2, 45.0, 45.2, 49.3, 50.6, 53.4, 57.6, 63.3, 65.6, 68.5, 69.0, 70.6, 72.5, 74.2, 76.5, 77.8, 78.1, 78.2, 80.8, 95.8, 102.5, 120.9, 128.6, 132.0, 133.5, 139.4, 175.4; HRMS (ESI) calc for [C<sub>46</sub>H<sub>73</sub>NO<sub>13</sub>+H]<sup>+</sup>+848.5155. found 848.5181

Descladinose-4'-Ethynylbenzylclarithromycin (5)

A solution of 4'-Ethynylbenzylclarithromycin 4 (27.3 g, 31.2 mmol) in EtOH (200 mL) and 1N HCL (200 mL) was stirred at room temperature for 17 h after which TLC analysis indicated the absence of starting material. The reaction mixture was basified with NH<sub>4</sub>OH to about pH 9, added H<sub>2</sub>O (250 mL) and EtOAc (350 mL) and separated the two layers. The aqueous layer was washed with EtOAc (250 mL) and the two organic layers combined and washed with saturated brine, then dried over Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified on silica column using gradient elution (12:1, 10:1 and 8:1 CH<sub>2</sub>Cl<sub>2</sub>/C<sub>2</sub>H<sub>5</sub>CO) to isolate 77% (17.1 g) of 5 as off-white foam.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.82 (3H, t, J=7.6 Hz), 1.09-1.28 (12H, m), 1.34 (3H, s), 1.40-1.55 (3H, m), 1.70-1.74 (2H, m), 1.87-1.94 (3H, m), 2.08-2.15 (6H, m), 2.54-

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2.66 (2H, m), 2.94-2.98 (3H, m), 3.05 (1H, s), 3.25 (1H, s), 3.31-3.42 (2H, m), 3.48-3.56 (2H, m), 3.66 (2H, d,  $J=10.0$  Hz), 3.82 (1H, s), 3.90 (1H, s), 4.35 (1H, d,  $J=7.6$  Hz), 5.14 (1H, dd,  $J=10.8, 2.0$  Hz), 7.18 (2H, d,  $J=8.0$  Hz), 7.42 (2H, d,  $J=8.4$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  8.4, 10.5, 12.7, 15.3, 16.3, 17.8, 18.8, 21.4, 29.2, 35.9, 36.6, 37.5, 38.7, 44.5, 45.5, 49.6, 57.8, 65.0, 69.7, 70.1, 70.6, 74.1, 77.9, 78.9, 83.3, 88.5, 106.5, 121.0, 128.4, 132.1, 139.1, 174.7; HRMS (ESI) calc for  $[\text{C}_{38}\text{H}_{59}\text{NO}_{10}+\text{H}]^+$ +690.4212. found 690.4259

## Synthesis of Compound (6) (Acetylation)

To a solution of descladinose-4'-ethynylbenzylclarithromycin 5 (3.8 g, 5.5 mmol) in acetone (20 ml),  $\text{Ac}_2\text{O}$  (0.62 g, 6.0 mmol) was added and stirred at  $40^\circ\text{C}$ . for 36 h. The reaction mixture was diluted with EtOAc (100 ml) and washed with aqueous  $\text{NaHCO}_3$  (70 ml) and saturated brine (70 ml). Purification on silica column (6:1  $\text{CH}_2\text{Cl}_2/\text{acetone}$ ) afforded 2.8 g (70%) of the title compound as a yellowish solid.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.80 (3H, t,  $J=7.2$  Hz), 0.90 (3H, d,  $J=7.2$  Hz), 1.08-1.47 (12H, m), 1.58 (6H, s), 1.63-2.05 (7H, m), 2.08 (3H, s), 2.16 (3H, s), 2.42-2.80 (3H, m), 2.92 (3H, s), 2.94-3.00 (1H, m), 3.03-3.68 (3H, m), 3.79 (2H, s), 3.94 (1H, s), 4.08 (1H, m), 4.54 (1H, d,  $J=8.0$  Hz), 4.80 (1H, m), 5.15 (1H, dd,  $J=11.6, 2.4$  Hz), 7.17 (2H, d,  $J=8.4$  Hz), 7.38 (2H, d,  $J=8.0$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  8.0, 10.7, 12.8, 15.5, 16.4, 18.1, 19.5, 21.3, 21.5, 21.6, 31.1, 31.9, 36.0, 37.0, 37.5, 38.7, 44.3, 45.7, 50.0, 58.4, 62.5, 68.9, 69.8, 71.6, 74.4, 76.9, 77.0, 77.8, 78.1, 81.3, 83.9, 99.9, 120.7, 128.4, 132.2, 141.1, 170.1, 174.9, 221.2; HRMS (FAB, mmba) calc for  $[\text{C}_{40}\text{H}_{61}\text{NO}_{11}+\text{H}]^+$ +732.43229. found 732.43105

## Synthesis of Compound (7) (Oxidation)

Methyl sulfide (1.42 g, 22.8 mmol), was added to a mixture of *N*-chlorosuccinimide (2.61 g, 19.5 mmol) and  $\text{CH}_2\text{Cl}_2$  (10 ml) while maintaining the temperature at  $-15^\circ\text{C}$ . Compound 6 (10.0 g, 13.7 mmol) dissolved in  $\text{CH}_2\text{Cl}_2$  (50 ml) was added to the reaction flask, followed by  $\text{Et}_3\text{N}$  (1.56 g, 15.4 mmol) and stirred at  $-15^\circ\text{C}$ . for 4.5 h. The reaction mixture was poured into EtOAc (350 ml) and 0.5 N aqueous NaOH (250 ml). The organic layer was separated and washed with saturated brine (250 ml), dried over  $\text{Na}_2\text{SO}_4$ , evaporated solvent and purified on silica column (2:3:0.1 EtOAc/Hexane/ $\text{Et}_3\text{N}$ ) to afford 9.54 g (96%) of the title compound as off-white solid.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.80-0.86 (6H, m), 1.09-1.57 (12H, m), 1.58 (6H, s), 1.62-2.02 (7H, m), 2.05 (3H, s), 2.15 (3H, s), 2.44-2.80 (2H, m), 2.92 (3H, s), 2.95-3.00 (1H, m), 3.05-3.66 (4H, m), 3.80 (1H, q,  $J=14.0, 6.8$  Hz), 3.89 (1H, s), 4.12 (1H, m), 4.38 (1H, d,  $J=8.0$  Hz), 4.79-4.83 (1H, m), 5.14 (1H, dd,  $J=11.2, 2.0$  Hz), 7.16 (2H, d,  $J=7.6$  Hz), 7.38 (2H, d,  $J=7.6$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  10.8, 12.4, 14.4, 14.5, 16.5, 17.9, 19.6, 21.3, 21.5, 31.3, 36.9, 37.6, 39.2, 45.1, 46.3, 49.7, 51.1, 58.5, 62.5, 69.2, 69.6, 71.5, 74.1, 77.0, 77.1, 77.2, 78.1, 83.9, 101.1, 120.7, 128.5, 132.2, 140.9, 169.6, 170.0, 205.7, 221.1; FIRMMS (FAB, mmba) calc for  $[\text{C}_{40}\text{H}_{59}\text{NO}_{11}+\text{H}]^+$ +730.41664. found 730.41321

## Carbamate Ketolide (8)

To a suspension of 7 (1.5 g, 2.06 mmol) in a mixture of anhydrous THF (20 ml) and anhydrous DMF (7 ml), was added CDI (1.3 g, 8.20 mmol) followed by a solution of  $\text{NaN}(\text{TMS})_2$  1.0 M in THF (2.6 ml, 2.6 mmol) over 75 min and stirred at RT for 24 h. The reaction mixture was diluted

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with EtOAc (50 ml), washed with aqueous  $\text{NaHCO}_3$  (20 ml) and saturated brine (20 ml) and dried over  $\text{Na}_2\text{SO}_4$ . The solvent was evaporated and purified on silica column (3:2:0.1 EtOAc/Hexane/ $\text{Et}_3\text{N}$ ) to yield 42% (0.7 g) of the title compound.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.91 (3H, t, 7.6 Hz), 1.09 (3H, d,  $J=7.2$  Hz), 1.14-1.50 (9H, m), 1.80 (3H, s), 1.83 (3H, s), 1.56-1.94 (7H, m), 2.00 (3H, s), 2.05 (3H, s), 2.12 (3H, s), 2.64-2.80 (1H, m), 3.02 (3H, s), 2.95-3.70 (4H, m), 3.73 (1H, q,  $J=14.0, 6.8$  Hz), 4.10-4.06 (2H, m), 4.30 (1H, d,  $J=7.6$  Hz), 4.77-4.80 (1H, m), 5.68 (1H, dd,  $J=11.2, 2.0$  Hz), 6.77 (1H, s), 7.03 (1H, s), 7.16 (2H, d,  $J=7.6$  Hz), 7.36 (3H, m), 8.06 (1H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  10.7, 13.5, 14.4, 15.2, 19.1, 20.4, 21.1, 21.2, 21.5, 22.8, 31.2, 36.8, 47.6, 50.5, 51.2, 58.5, 60.6, 62.8, 69.3, 71.6, 78.7, 83.9, 84.7, 117.3, 120.7, 128.4, 131.1, 132.2, 137.3, 138.8, 140.9, 146.2, 169.1, 169.8; HRMS (FAB, mmba) calc for  $[\text{C}_{44}\text{H}_{59}\text{N}_3\text{O}_{11}+\text{H}]^+$ +806.42279. found 806.42103

## Tricyclic Ketolide (9)

To a solution of 8 (0.6 g, 0.74 mmol) in  $\text{CH}_3\text{CN}$  (10 ml) and  $\text{H}_2\text{O}$  (1 ml), was added ethylenediamine (0.44 g, 7.40 mmol) and heated in a sealed tube at  $50-55^\circ\text{C}$ . for 24 h. The solvent was evaporated off and the reaction mixture partitioned between  $\text{H}_2\text{O}$  (15 ml) and  $\text{CH}_2\text{Cl}_2$  (20 ml) and separated. The organic layer was washed with saturated brine (20 ml), dried over  $\text{Na}_2\text{SO}_4$ , and the solvent evaporated. The crude product was dissolved in anhydrous MeOH (15 ml) and heated at  $90^\circ\text{C}$ . in a sealed tube for 24 h. MeOH was evaporated and the product purified on silica column (EtOAc/ $\text{Et}_3\text{N}$  12:0.1). EtOH (6 ml) followed by AcOH (0.043 g, 0.71 mmol) were added to the pure product (0.27 g, 0.34 mmol) and heated at  $95^\circ\text{C}$ . in a sealed tube for 20 h. The reaction mixture was suspended in dilute  $\text{NH}_4\text{OH}$  (10 ml) and  $\text{CH}_2\text{Cl}_2$  (15 ml) and separated the organic layer, washed with saturated brine (10 ml), and dried over  $\text{Na}_2\text{SO}_4$ . Purification on silica column (EtOAc/MeOH/ $\text{Et}_3\text{N}$  10:0.5:0.05) afforded 0.24 g (57%) of compound 9.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.80 (3H, t,  $J=7.2$  Hz), 1.00 (3H, d,  $J=6.8$  Hz), 1.14-1.55 (12H, m), 1.30 (3H, s), 1.43 (3H, s), 1.60-1.96 (6H, m), 2.12 (3H, s), 2.45-2.95 (6H, m), 3.03 (3H, s), 3.23-3.43 (4H, m), 3.64-3.78 (4H, m), 3.94 (1H, m), 4.16 (1H, d,  $J=8.4$  Hz), 4.25 (1H, d,  $J=7.6$  Hz), 4.90 (1H, d,  $J=10.0$  Hz), 7.18 (2H, d, 8.0 Hz), 7.39 (2H, d,  $J=7.2$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  10.6, 11.1, 13.0, 14.6, 16.6, 19.3, 19.8, 21.4, 22.3, 29.7, 36.5, 37.1, 38.7, 42.5, 43.0, 48.3, 49.3, 49.7, 51.4, 57.6, 60.1, 65.6, 69.7, 70.5, 76.7, 78.7, 79.4, 81.7, 83.6, 104.0, 121.0, 128.8, 132.4, 140.0, 156.3, 169.8, 181.5, 204.4; HRMS (FAB, mmba) calc for  $[\text{C}_{41}\text{H}_{59}\text{N}_3\text{O}_9+\text{H}]^+$ +738.43296. found 738.43318

Azidoalkyl-O-trityl Hydroxamate Derivatives  
(10a-e)

## Method A

Tricyclic Ketolide-N-benzyltriazolylhexa-N-Otrityl (11a) General Procedure: 4'-Ethynylbenzyl tricyclic ketolide 9 (0.105 g, 0.142 mmol) and 6-azidohexa-o-trityl hydroxamic acid 10a (0.058 g, 0.142 mmol) were dissolved in anhydrous THF (7 mL) and stirred under argon at room temperature. Copper (I) iodide (0.012 g, 0.063 mmol) and Hunig's base (0.6 mL) were added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with 1:4  $\text{NH}_4\text{OH}/\text{saturated NH}_4\text{Cl}$  (50 mL) and extracted with 10% MeOH/ $\text{CH}_2\text{Cl}_2$  (3x30 mL). The organic layers were combined, dried over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo. The

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crude product was purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) to give 127 mg (78%) of 11a as a yellowish solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.85 (3H, t, J=7.6 Hz), 1.1 (3H, d, J=6.8 Hz), 1.20-1.39 (17H, m), 1.46-2.00 (15H, m), 2.19 (3H, s), 2.58-2.80 (6H, m), 2.84-3.00 (1H, m), 3.02-3.17 (1H, m), 3.31-3.60 (3H, m), 3.64-3.87 (5H, m), 3.96-4.0 (1H, m), 4.22 (1H, d, J=8.4 Hz), 4.29-4.32 (3H, m), 4.93 (1H, dd, J=10.0, 1.6 Hz), 7.23-7.45 (17H, m), 7.71 (1H, s), 7.79 (2H, d, J=8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.6, 16.7, 19.3, 19.9, 21.4, 22.3, 22.8, 26.0, 29.6, 30.2, 31.0, 36.5, 37.0, 38.8, 42.5, 43.0, 48.3, 49.3, 49.7, 50.2, 51.4, 53.7, 57.7, 60.1, 65.6, 69.8, 70.5, 76.7, 78.7, 79.4, 81.8, 93.1, 104.1, 119.7, 126.0, 128.2, 128.4, 129.2, 129.4, 130.0, 138.9, 141.2, 147.6, 156.3, 169.8, 177.0, 181.5, 204.5; HRMS (ESI) calc for [C<sub>66</sub>H<sub>85</sub>N<sub>7</sub>O<sub>11</sub>+H]<sup>+</sup> 1152.6379. found 1152.6367

Tricyclic Ketolide-N-benzyltriazolylhepta-N-Otrityl  
(11b)

Reaction of 4'-ethynylbenzyl tricyclic ketolide 9 (0.102 g, 0.138 mmol) and 6-azidohepta-o-trityl hydroxamic acid 10b (0.059 g, 0.138 mmol) within 24 h as described for the synthesis and purification of 11a gave 133 mg (83%) of 11b as a yellowish solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.85 (3H, t, J=7.6 Hz), 1.06 (3H, d, J=6.8 Hz), 1.20-1.42 (19H, m), 1.45-1.98 (15H, m), 2.20 (3H, s), 2.57-2.79 (6H, m), 2.85-3.00 (1H, m), 3.03-3.17 (1H, m), 3.30-3.57 (3H, m), 3.72-3.83 (5H, m), 3.96-4.00 (1H, m), 4.22 (1H, d, J=8.8 Hz), 4.29-4.35 (3H, m), 4.95 (1H, dd, J=10.4, 2.4 Hz), 7.22-7.45 (17H, m), 7.71 (1H, s), 7.78 (2H, d, J=8.4 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.6, 16.6, 19.3, 19.9, 21.4, 22.3, 26.3, 29.6, 30.2, 36.5, 37.0, 38.8, 42.5, 43.0, 48.3, 49.3, 49.7, 50.4, 51.4, 53.7, 57.7, 60.1, 65.5, 69.7, 70.5, 76.7, 78.7, 79.4, 81.8, 92.8, 104.1, 119.7, 126.0, 128.4, 129.2, 129.4, 130.0, 132.3, 138.9, 141.3, 147.6, 156.3, 169.8, 177.6, 182.5, 204.5; HRMS (ESI) calc for [C<sub>67</sub>H<sub>87</sub>N<sub>7</sub>O<sub>11</sub>+H]<sup>+</sup> 1166.6536. found 1166.6478

Tricyclic Ketolide-N-benzyltriazolyl octa-N-Otrityl  
(11c)

Reaction of 4'-ethynylbenzyl tricyclic ketolide 9 (0.075 g, 0.102 mmol) and 6-azido octa-o-trityl hydroxamic acid 10c (0.045 g, 0.102 mmol) in anhydrous THF (5 ml) within 22 h as described for the synthesis and purification of 11a gave 94 mg (79%) of 11c as a yellowish solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83 (3H, t, J=7.2 Hz), 1.04 (3H, d, J=7.2 Hz), 1.15-1.41 (21H, m), 1.45-1.95 (15H, m), 2.18 (3H, s), 2.55-2.73 (6H, m), 2.87-2.97 (1H, m), 3.03-3.10 (1H, m), 3.28-3.57 (3H, m), 3.70-3.82 (5H, m), 3.93-3.97 (1H, m), 4.21 (1H, d, J=8.4 Hz), 4.27-4.34 (3H, m), 4.94 (1H, dd, J=12.8, 2.4 Hz), 7.25-7.42 (17H, m), 7.71 (1H, s), 7.76 (2H, d, J=8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.6, 16.6, 19.3, 19.9, 21.4, 22.3, 26.4, 28.8, 29.6, 30.4, 36.6, 37.0, 38.8, 42.5, 42.9, 48.3, 49.3, 49.7, 50.6, 51.4, 53.7, 57.7, 60.1, 65.5, 69.8, 70.5, 76.7, 78.7, 79.4, 81.8, 93.8, 104.1, 119.6, 126.0, 127.7, 128.3, 128.8, 129.2, 129.4, 130.0, 132.7, 138.9, 141.3, 147.7, 156.3, 169.8, 177.0, 182.4, 204.4; HRMS (ESI) calc for [C<sub>68</sub>H<sub>89</sub>N<sub>7</sub>O<sub>11</sub>+H]<sup>+</sup> 1180.6692. found 1180.6637

Tricyclic Ketolide-N-benzyltriazolyl nona-N-Otrityl  
(11d)

Reaction of 4'-ethynylbenzyl tricyclic ketolide 9 (0.075 g, 0.102 mmol) and 6-azidonona-o-trityl hydroxamic acid 10d (0.046 g, 0.102 mmol) in anhydrous THF (5 ml) within 20 h

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as described for the synthesis and purification of 11a gave 93 mg (76%) of 11d as a yellowish solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.85 (3H, t, J=8.0 Hz), 1.05 (3H, d, J=6.8 Hz), 1.16-1.44 (23H, m), 1.45-1.96 (15H, m), 2.20 (3H, m), 2.57-2.78 (6H, m), 2.90-2.99 (1H, m), 3.02-3.12 (1H, m), 3.30-3.57 (3H, m), 3.72-3.84 (5H, m), 3.96-4.00 (1H, m), 4.29 (1H, d, J=8.4 Hz), 4.31 (1H, d, J=7.2 Hz), 4.37 (2H, t, J=7.2 Hz), 4.95 (1H, dd, J=12.8, 2.4 Hz), 7.19-7.45 (17H, m), 7.72 (1H, s), 7.78 (2H, d, J=8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.6, 16.6, 19.3, 19.9, 21.4, 22.3, 26.6, 28.9, 29.2, 29.6, 29.9, 30.5, 36.5, 37.0, 37.1, 38.8, 42.5, 43.0, 48.3, 49.3, 49.7, 50.6, 51.4, 57.7, 60.1, 65.5, 65.6, 69.7, 70.5, 76.7, 78.7, 79.4, 81.8, 94.3, 104.1, 119.6, 126.0, 128.3, 128.8, 129.0, 129.2, 129.4, 130.0, 132.3, 138.9, 141.3, 147.6, 156.3, 169.8, 178.0, 181.5, 204.5; HRMS (ESI) calc for [C<sub>69</sub>H<sub>91</sub>N<sub>7</sub>O<sub>11</sub>+H]<sup>+</sup> 1194.6849. found 1194.6838

Tricyclic Ketolide-N-benzyltriazolyl deca-N-Otrityl  
(11e)

Reaction of 4'-ethynylbenzyl tricyclic ketolide 9 (0.075 g, 0.102 mmol) and 6-azidodeca-o-trityl hydroxamic acid 10e (0.048 g, 0.102 mmol) in anhydrous THF (5 ml) within 20 h as described for the synthesis and purification of 11a gave 98 mg (80%) of 11e as a yellowish solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83 (3H, t, J=6.8 Hz), 1.03 (3H, d, J=7.2 Hz), 1.15-1.40 (25H, m), 1.44-1.96 (15H, m), 2.17 (3H, s), 2.57-2.78 (6H, m), 2.87-2.95 (1H, m), 3.03-3.08 (1H, m), 3.28-3.53 (3H, m), 3.70-3.82 (5H, m), 3.92-3.96 (1H, m), 4.20 (1H, d, J=8.4 Hz), 4.29 (1H, d, J=6.8 Hz), 4.33 (2H, t, J=7.2 Hz), 4.93 (1H, d, J=10.0 Hz), 7.26-7.43 (17H, m), 7.73 (1H, s), 7.76 (2H, d, J=8.0 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.6, 16.6, 19.3, 19.8, 21.4, 22.3, 26.6, 29.1, 29.3, 29.6, 29.9, 30.5, 36.6, 37.0, 38.8, 42.5, 43.0, 48.3, 49.3, 49.7, 50.6, 51.4, 57.7, 60.1, 65.5, 69.7, 70.5, 76.7, 78.7, 79.4, 81.8, 93.4, 104.1, 199.6, 126.0, 128.3, 129.2, 129.4, 130.0, 138.9, 147.6, 156.3, 169.8, 177.6, 182.0, 204.5; HRMS (ESI) calc for [C<sub>70</sub>H<sub>93</sub>N<sub>7</sub>O<sub>11</sub>+H]<sup>+</sup> 1208.7005. found 1208.6888

Tricyclic Ketolide-N-benzyltriazolyl hexahydroxamic acid (56a)

Protocol B: To a solution of tricyclic ketolide-N-benzyltriazolyl hexa-N-Otrityl 11a (0.120 g, 0.105 mmol) in methylene chloride (1 ml) at 0° C., was added thioanisole (0.2 ml) and TFA (0.2 ml) dropwise. Stirring was continued at 0° C. for 2 h after which TLC analysis indicated completion of reaction. Excess TFA and solvent were evaporated off and immediately placed back in the ice-bath followed by addition of PBS buffer (10 ml). Saturated NaHCO<sub>3</sub> was added dropwise until the pH was neutral. Extraction with 20% MeOH/CH<sub>2</sub>Cl<sub>2</sub> (10 ml×3), drying over Na<sub>2</sub>SO<sub>4</sub>, and evaporation of solvent afforded liquid crude product which was then purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) to afford 47 mg (50%) of 56a as a yellowish solid.

Protocol C: To a mixture of tricyclic ketolide-N-benzyltriazolyl hexa-N-Otrityl 11a (0.136 g, 0.118 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/MeOH (2 ml: 2 ml) was added BF<sub>3</sub>·OEt<sub>2</sub> (0.033 g, 0.236 mmol) and stirred at room temperature for 45 min. Dilute NaHCO<sub>3</sub> (20 ml) was added until pH=8, and then extracted with 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub> (10 ml×4), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) to give 64 mg (60%) of 56a as a yellowish solid.

Protocol D: 4'-Ethynylbenzyl tricyclic ketolide 9 (0.050 g, 0.065 mmol) and 6-azidohexahydroxamic acid 13a (0.025 g, 0.145 mmol) were dissolved in anhydrous THF (7 mL) and

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stirred under argon at room temperature. Copper (I) iodide (0.007 g, 0.036 mmol) and Hunig's base (0.5 mL) were added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and washed with 1:4 NH<sub>4</sub>OH/saturated NH<sub>4</sub>Cl (3×10 mL) and saturated NH<sub>4</sub>Cl (10 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The crude product was purified by prep TLC (silica, 12:1:0.1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH/conc. NH<sub>4</sub>OH) to give 20 mg (34%) of 56a as a brown solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.85 (3H, t, J=8.0 Hz), 1.05 (3H, d, J=7.2 Hz), 1.20-2.00 (30H, m), 2.13-2.18 (2H, m), 2.20 (3H, s), 2.58-2.72 (6H, m), 2.92-2.98 (1H, m), 3.05-3.09 (1H, m), 3.30-3.55 (3H, m), 3.70-3.84 (5H, m), 3.95-3.98 (1H, m), 4.22 (1H, d, J=8.0 Hz), 4.31-4.38 (3H, m), 4.95 (1H, d, J=8.8 Hz), 7.33 (2H, d, J=8.0 Hz), 7.77 (3H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.6, 11.1, 13.1, 14.6, 16.5, 19.3, 19.8, 21.4, 22.3, 24.7, 25.9, 29.7, 29.9, 30.0, 36.6, 37.1, 38.8, 42.5, 42.8, 49.4, 49.6, 50.3, 51.4, 53.7, 57.7, 60.1, 65.5, 69.7, 70.5, 76.7, 78.7, 79.3, 81.8, 104.1, 120.1, 126.0, 129.5, 129.7, 139.1, 147.7, 156.3, 169.8, 171.1, 182.3, 204.5; HRMS (ESI) calc for [C<sub>47</sub> H<sub>71</sub> N<sub>7</sub> O<sub>11</sub>+H]<sup>+</sup> 910.5284. found 910.5279; Melting point 127-130° C.

Tricyclic  
Ketolide-N-benzyltriazolylheptahydroxamic acid  
(56b)

According to protocol B, reaction of 11b (0.132 g, 0.113 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), thioanisole (0.3 mL) and TFA (0.3 mL) for 3 h afforded 56 mg (55%) of 56b as a yellowish solid.

According to protocol C, reaction of 11b (0.15 g, 0.127 mmol) and BF<sub>3</sub>·OEt<sub>2</sub> (0.037 g, 0.257 mmol) afforded 65 mg (55%) of 56b as a yellowish solid.

Reaction of 4'-ethynylbenzyl tricyclic ketolide 9 (0.048 g, 0.063 mmol) and 7-azidoheptahydroxamic acid 13b (0.020 g, 0.107 mmol) within 12 h, according to the protocol D described for the synthesis of compound 56a, gave 22 mg (38%) of 56b as a brown solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83 (3H, t, J=7.6 Hz), 1.03 (3H, d, J=6.4 Hz), 1.18-1.90 (32H, m), 2.10-2.13 (2H, m), 2.17 (3H, s), 2.58-2.73 (6H, m), 2.93-2.98 (1H, m), 3.03-3.07 (1H, m), 3.28-3.60 (3H, m), 3.70-3.81 (5H, m), 3.98 (1H, m), 4.20 (1H, d, J=8.4 Hz), 4.29-4.38 (3H, m), 4.93 (1H, d, J=10.4 Hz), 7.30 (2H, d, J=7.6 Hz), 7.75 (2H, d, J=7.6 Hz), 7.78 (1H, s); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.6, 11.1, 13.1, 14.6, 16.5, 19.3, 19.8, 21.4, 22.3, 25.2, 26.0, 28.2, 29.7, 29.9, 30.1, 36.6, 37.0, 38.8, 42.5, 42.8, 49.3, 49.5, 50.4, 51.4, 53.7, 57.7, 60.1, 65.5, 69.7, 70.5, 76.7, 78.7, 79.3, 81.8, 104.0, 120.0, 126.0, 129.4, 129.8, 139.1, 147.7, 156.3, 169.8, 171.2, 182.4, 204.5; HRMS (ESI) calc for [C<sub>48</sub> H<sub>73</sub> N<sub>7</sub> O<sub>11</sub>+H]<sup>+</sup> 924.5440. found 924.5422; Melting point 128-131° C.

Tricyclic Ketolide-N-benzyltriazolyldecahydroxamic acid (56c)

Reaction of 11c (0.092 mg, 0.079 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), thioanisole (0.2 mL) and TFA (0.2 mL) according to protocol B afforded 37 mg (51%) of 56c as a yellowish solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83 (3H, t, J=6.4 Hz), 1.03 (3H, d, J=5.6 Hz), 1.18-1.60 (21H, m), 1.66-2.1 (15H, m), 2.18 (3H, s), 2.56-2.70 (6H, m), 2.90-2.98 (1H, m), 3.02-3.07 (1H, m), 3.28-3.58 (3H, m), 3.70-3.81 (5H, m), 3.94-3.97 (1H, m), 4.20 (1H, d, J=7.2 Hz), 4.28 (1H, d, J=6.0 Hz), 4.35 (2H, m), 4.93 (1H, d, J=10.4 Hz), 7.30 (2H, d, J=7.2 Hz), 7.73-7.76 (3H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.6, 11.1, 13.1, 14.4, 14.6, 16.5, 19.3, 19.8, 21.3, 21.4, 22.3, 25.2, 26.1,

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28.3, 29.7, 29.9, 30.2, 32.9, 36.6, 37.1, 38.8, 42.5, 42.7, 48.2, 49.4, 49.5, 50.5, 51.4, 57.7, 60.1, 60.6, 65.5, 69.7, 70.5, 76.7, 78.7, 79.3, 81.8, 104.0, 119.9, 126.0, 129.5, 129.8, 139.0, 147.7, 156.3, 169.8, 171.7, 182.4, 204.5; HRMS (ESI) calc for [C<sub>49</sub>H<sub>75</sub> N<sub>7</sub> O<sub>11</sub>+H]<sup>+</sup> 938.5597. found 938.5556; Melting point 113-115° C.

Tricyclic Ketolide-N-benzyltriazolylnonahydroxamic acid (56d)

Reaction of 11d (0.091 mg, 0.077 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), thioanisole (0.2 mL) and TFA (0.2 mL) according to protocol B afforded 43 mg (60%) of 56d as a yellowish solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83 (3H, t, J=7.2 Hz), 1.03 (3H, d, J=7.2 Hz), 1.17-1.60 (23H, m), 1.66-2.14 (15H, m), 2.17 (3H, s), 2.58-2.73 (6H, m), 2.87-2.95 (1H, m), 3.00-3.07 (1H, m), 3.27-3.55 (3H, m), 3.70-3.82 (5H, m), 3.93-3.97 (1H, m), 4.20 (1H, d, J=8.8 Hz), 4.28 (1H, d, J=7.2 Hz), 4.35 (2H, t, J=6.8 Hz), 4.92 (1H, dd, J=10.0, 1.2 Hz), 7.30 (2H, d, J=8.0 Hz), 7.75 (3H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.6, 16.5, 19.3, 19.8, 21.4, 22.3, 25.4, 26.3, 28.6, 28.8, 28.9, 29.6, 29.9, 30.3, 32.9, 36.6, 37.1, 38.8, 42.5, 42.8, 48.2, 49.3, 49.6, 50.6, 51.4, 57.7, 60.1, 65.5, 69.7, 70.5, 76.7, 78.7, 79.3, 81.8, 104.1, 119.8, 126.0, 129.5, 129.8, 139.0, 147.7, 156.3, 169.8, 171.3, 182.9, 204.5; HRMS (ESI) calc for [C<sub>50</sub> H<sub>77</sub> N<sub>7</sub> O<sub>11</sub>+H]<sup>+</sup> 952.5753. found 952.5728; Melting point 112-115° C.

Tricyclic Ketolide-N-benzyltriazolyldecahydroxamic acid (56e)

Reaction of 11e (0.097 mg, 0.081 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL), thioanisole (0.2 mL) and TFA (0.2 mL) according to protocol B afforded 42 mg (55%) of 56e as a yellowish solid.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 0.83 (3H, t, J=7.2 Hz), 1.03 (3H, d, J=6.4 Hz), 1.18-1.61 (25H, m), 1.66-2.14 (15H, m), 2.18 (3H, s), 2.59-2.73 (6H, m), 2.89-2.94 (1H, m), 3.01-3.07 (1H, m), 3.28-3.54 (3H, m), 3.70-8.83 (5H, m), 3.93-3.97 (1H, m), 4.20 (1H, d, J=8.4 Hz), 4.28 (1H, d, J=6.8 Hz), 4.36 (2H, J=7.2 Hz), 4.92 (1H, dd, J=10.4, 1.6 Hz), 7.30 (2H, d, J=8.0 Hz), 7.76 (3H, m); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 10.7, 11.1, 13.1, 14.4, 14.6, 16.5, 19.3, 19.9, 21.4, 22.3, 25.5, 26.3, 28.7, 28.9, 29.0, 29.6, 29.9, 30.3, 33.1, 36.6, 37.1, 38.8, 42.5, 42.7, 48.2, 49.4, 49.5, 50.6, 51.4, 57.7, 60.1, 60.6, 65.5, 69.7, 70.5, 76.7, 78.7, 79.3, 81.8, 104.0, 119.8, 126.0, 129.5, 129.8, 139.0, 147.7, 156.3, 169.8, 171.7, 182.9, 204.5; HRMS (ESI) calc for [C<sub>51</sub> H<sub>79</sub> N<sub>7</sub> O<sub>11</sub>+H]<sup>+</sup> 966.5910. found 966.5919; Melting point 123-126° C.

## Example 5

Anti-HDAC Activity of Nonpeptide Macrocyclic HDAC Inhibitors

Inhibition of HeLa nuclear extract HDAC 1/2 and HDAC8 by compounds 7-14, 23-30, 36, and 38 was evaluated in a Fluor de Lys assay according to the manufacture's protocol. Each IC<sub>50</sub> value was obtained by averaging three independent experiments. This data is shown in Table 4. The compounds displayed both linker-length and macrolide-type dependent HDAC inhibition activities with IC<sub>50</sub> in low nanomolar range.



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TABLE 4

Inhibitory activity of HDAC inhibitors		
Compound	HDAC 1/2 (nM)	HDAC 8 (nM)
7	91.6	4,730
8	88.8	3,740
9	13.85	994
10	10.56	1,020
11	58.88	7,130
12	72.44	6,780
13	145.50	11,050
14	226.73	N.D.
23	36.98	3,990
24	44.26	4,750
25	4.09	1,890
26	1.87	1,390
27	55.59	5,880
28	123.03	4,420
29	169.80	10,550
30	223.36	N.D.
36	107.1	6,680
38	109.8	2,320

The ketolides 56 were also evaluated for their HDAC inhibitory activity against HDACs 1 and 2 from HeLa cell nuclear extract, HDAC 6, and HDAC 8 using the Fluor de Lys assay. The results are shown in Table 5.

TABLE 5

HDAC inhibition activity (IC <sub>50</sub> ) and Isoform Selectivity of tricyclic ketolide-based HDAC inhibitors						
Compd	n	Nuclear Extract (nM)	HDAC 8 (nM)	HDAC8: Nuclear Extract Isoform Selectivity	HDAC 6 (nM)	HDAC6: Nuclear Extract Isoform Selectivity
56a	1	7.77	796.2	102.5	1180.1	151.9
56b	2	1.03	544.6	528.7	728.7	707.5
56c	3	104.2	1909.3	18.3	1709.8	16.4
56d	4	163.6	2859.9	17.5	1916.9	11.7
56e	5	208.2	4557.8	21.9	3203.1	15.4

Results show a linker-dependent anti-HDAC activity which peaked with compound 56b, an analog having 6 methylene spacers separating the triazole ring from the zinc binding hydroxamic acid group (Table 5). Compound 56b potently inhibits the deacetylase activity of HDACs 1 and 2 with a single digit nanomolar IC<sub>50</sub>. Compounds 56c-e, analogs having longer methylene spacers than those of 56b, show a progressive reduction in anti-HDAC activity with increase in methylene spacer length. The HDAC inhibition profiles of these triketolide-derived HDACi paralleled those we previously reported for the macrolide-derived HDACi.

To obtain evidence for the HDAC isoform selectivity, the triketolide HDAC inhibitors were tested against HDAC 6 and HDAC 8. Compared to SAHA, compounds 56a and 56b are more selective for HDAC 1/2 with selectivity indices comparable to those of their 14-membered macrolide congeners. Compounds 56c-e are less selective for either HDAC isoform compared to 56a-b. However, 56c-e have improved HDAC 6 and comparable HDAC 8 isoform selectivity relative to SAHA (Table 5).

#### Example 6

##### Evaluating In Vitro Anti-Cancer Activity of HDAC Inhibitors

The potency of compounds in Table 5 were investigated by determining the drug concentrations necessary for 50% inhi-

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bition of cell viability (IC<sub>50</sub>) in SKMES 1, NCI-H69, DU 145 cells, lung fibroblasts, and HMEC. Drug concentrations necessary for 50% inhibition of cell viability (EC<sub>50</sub>) were quantitatively measured using trypan blue exclusion according to literature protocol (Mosmann, T. (1983) *J. Immunol. Methods* 65: 55; Chen et al. (2008) *Bioorg. Med. Chem.* 16: 4839). Table 6 shows the EC<sub>50</sub> values for each compound. All compounds inhibit the proliferation of the transformed cells studied with EC<sub>50</sub> in low micromolar range. Most importantly, these compounds are less toxic to untransformed cell-lines (lung fibroblast and HMEC) that we have studied to date.

TABLE 6

Cell growth inhibitory data					
Compound	SKMES 1 (uM)	NCI-H69 (uM)	DU-145 (uM)	Lung fibroblast (uM)	HMEC (uM)
7	1.79	1.92	1.45	>10	>10
8	1.68	1.77	1.24	>10	>10
9	2.33	3.45	1.88	>10	>10
10	2.56	3.01	1.97	>10	>10
11	4.89	4.56	5.89	>10	>10
12	4.67	3.99	5.68	>10	>10

TABLE 6-continued

Cell growth inhibitory data					
Compound	SKMES 1 (uM)	NCI-H69 (uM)	DU-145 (uM)	Lung fibroblast (uM)	HMEC (uM)
13	7.54	8.45	>10	>10	>10
23	2.15	2.67	2.98	>10	>10
24	1.95	1.92	3.29	>10	>10
25	1.33	1.45	1.12	>10	>10
26	1.28	1.49	1.05	>10	>10
27	4.89	5.67	6.97	>10	>10
28	4.45	5.09	5.78	>10	>10
29	7.12	7.29	8.14	>10	>10

The effect of compounds 56a-e on cancer cell lines: prostate (DU-145), lung (A549), and breast cancer (MCF-7) and a non-transformed cell, human lung fibroblast cell (Hs1.Lu) were also evaluated. Drug concentrations necessary for 50% inhibition of cell viability (EC<sub>50</sub>) were quantitatively measured using MTS colorimetric assay as described above. Table 7 shows the EC<sub>50</sub> values of each compound.

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TABLE 7

Anti-proliferative activity ( $\mu\text{M}$ ) of tricyclic ketolide-based HDAC inhibitors					
Compd	n	DU-145 ( $\mu\text{M}$ )	A549 ( $\mu\text{M}$ )	MCF-7 ( $\mu\text{M}$ )	Hs1.Lu Normal Lung Fibroblast ( $\mu\text{M}$ )
56a	1	1.64	1.17	1.26	N.D.
56b	2	0.82	0.66	0.75	N.D.
56c	3	3.70	1.64	1.35	N.D.
56d	4	3.89	2.48	2.56	N.D.
56e	5	4.83	2.81	3.68	N.D.

Compounds 56a-e inhibit the proliferation of all transformed cells studied with anti-proliferative activity that closely matched their in vitro ant-HDAC activity. Specifically, compound 56b has the most potent anti-proliferative activity with high nanomolar efficacy against all three cancer cell lines. Moreover, none of the compound shows any discernable toxicity against normal Hs1.Lu cells at drug concentrations in excess of 10  $\mu\text{M}$  (Table 7). These data showed that the triketolide hydroxamates reported here are selectively toxic to the transformed cells.

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Example 7

### Antiparasitic Activity of Non-Peptide Macrocyclic Histone Deacetylase Inhibitors

Macrocyclic histone deacetylase inhibitors were evaluated for parasitic activity against *Plasmodium falciparum* and *Leishmania donovani*. *P. falciparum* and *L. donovani* are the causative parasites of malaria and leishmaniasis, two human diseases which constitute a serious threat to public health in tropical and sub-tropical countries. Antimalarial activity was evaluated in vitro using chloroquine-sensitive (D6, Sierra Leone) and chloroquine-resistant (W2, Indochina) strains of *P. falciparum*. Antileishmanial activity was evaluated in vitro against the promastigote stage of *L. donovani*.

*Plasmodium* growth inhibition was determined by a parasite lactate dehydrogenase assay using Malstat reagent. Inhibition of viability of the promastigote stage of *L. donovani* was determined using standard Alamar blue assay, modified to a fluorometric assay. Amphotericin B and pentamidine, standard antileishmanial agents; chloroquine and artemisinin, standard antimalarial; and suberoylanilide hydroxamic acid (SAHA), standard HDACi were all used as positive controls. To determine selective toxicity index, all compounds were tested against nontransformed mammalian cell lines namely, monkey kidney fibroblasts (Vero) and murine macrophages (J774.1) using Neutral Red assay. The results are shown in Table 8.

TABLE 8

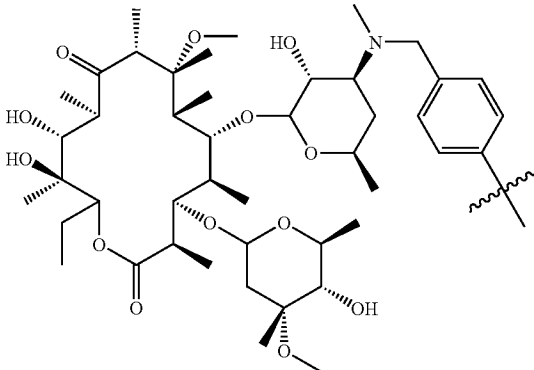
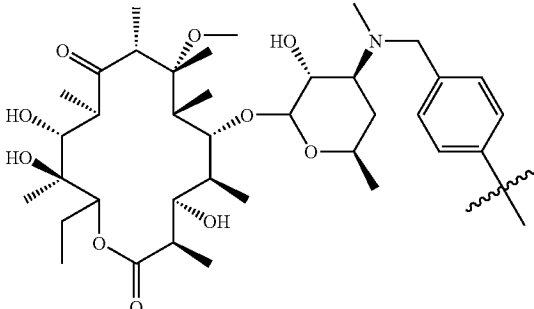
Antiparasitic activity of non-peptide HDAC inhibitors							
Compd.	R	HDAC n	Inhibition IC <sub>50</sub> (nM) <sup>3</sup>	Antimalarial Activity			
				Antileishmanial activity		<i>Plasmodium falciparum</i>	<i>Plasmodium falciparum</i>
				IC <sub>50</sub> (μg/ml)	IC <sub>90</sub> (μg/ml)	(D6 clone) IC <sub>50</sub> (μg/ml)	(W2 clone) IC <sub>50</sub> (μg/ml)
1		5	37.0	NA	NA	0.90	0.93
2		5	44.3	NA	NA	1.20	1.40

TABLE 8-continued

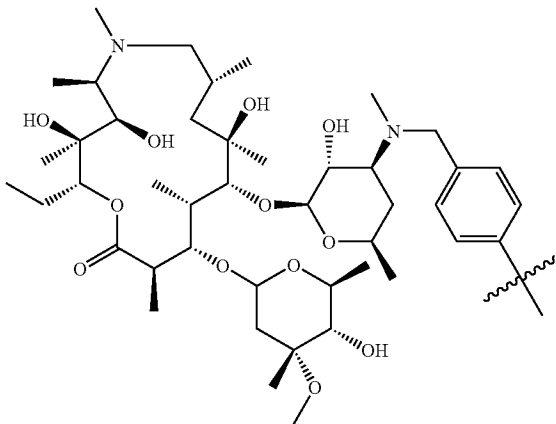
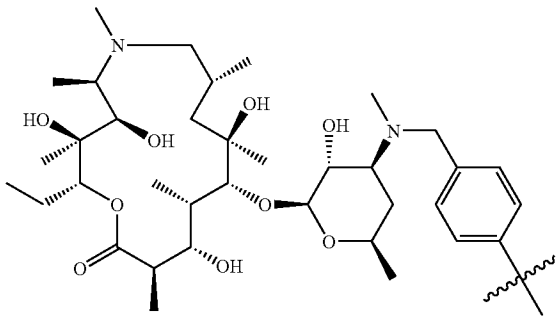
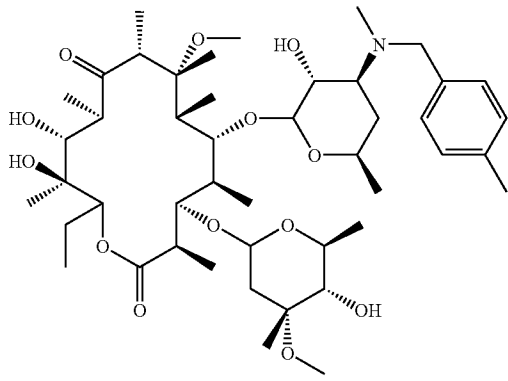
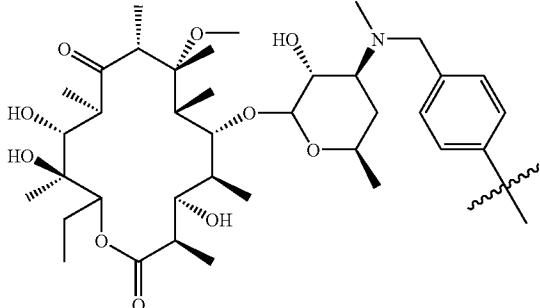
Antiparasitic activity of non-peptide HDAC inhibitors							
3		5	91.6	NA	NA	1.20	1.60
4		5	88.8	NA	NA	1.30	1.70
5		6	4.1	18	NA	0.27	0.31
6		6	1.9	NA	NA	0.18	0.25

TABLE 8-continued

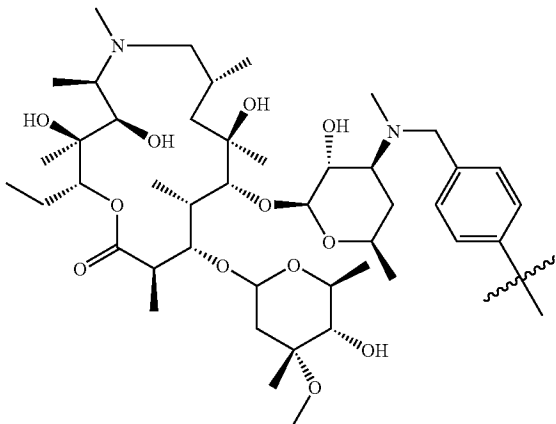
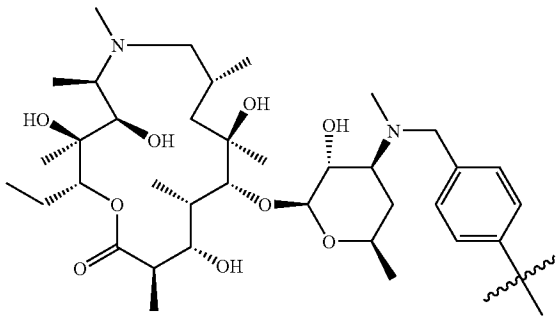
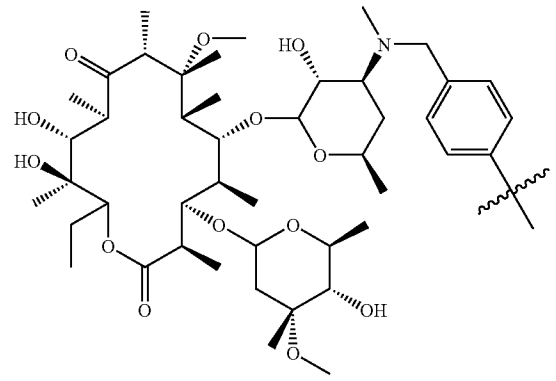
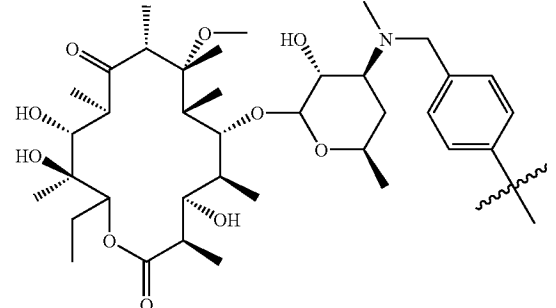
Antiparasitic activity of non-peptide HDAC inhibitors							
7		6	13.9	NA	NA	0.23	0.10
8		6	10.6	NA	NA	0.16	0.17
9		7	55.6	20	34	2.50	2.0
10		7	123.0	NA	NA	3.00	2.70

TABLE 8-continued

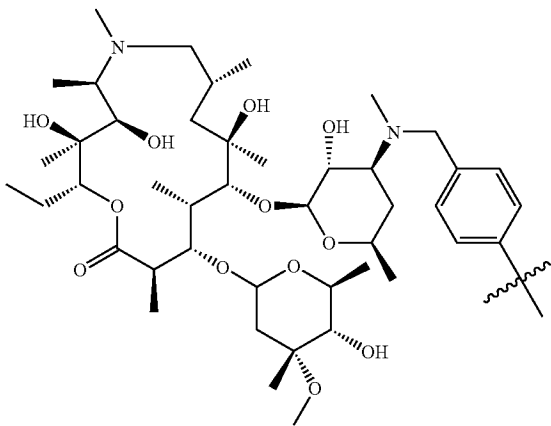
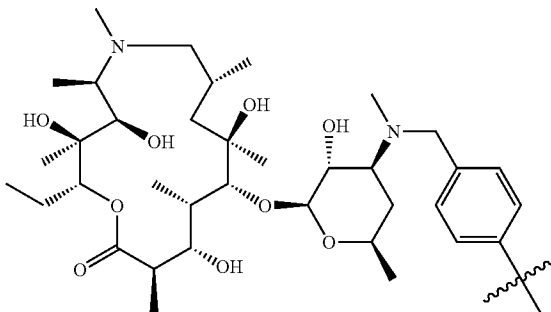
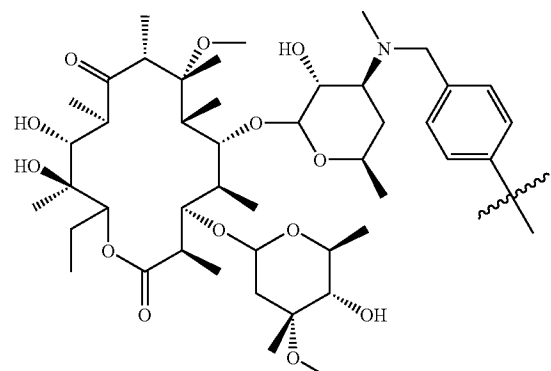
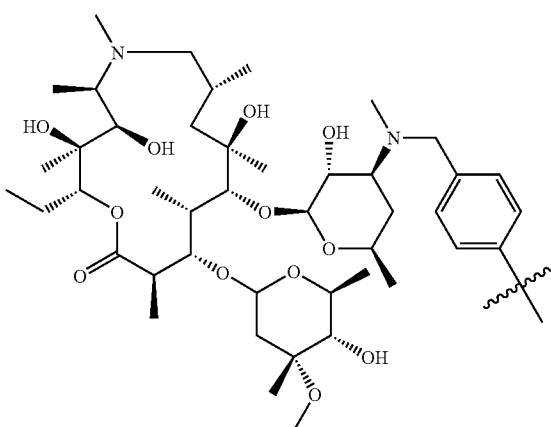
Antiparasitic activity of non-peptide HDAC inhibitors							
11		7	58.9	NA	NA	2.40	2.30
12		7	72.4	NA	NA	3.5	3.2
13		8	169.8	3.40	7.00	1.7	1.00
14		8	145.5	20	35	1.10	0.83

TABLE 8-continued

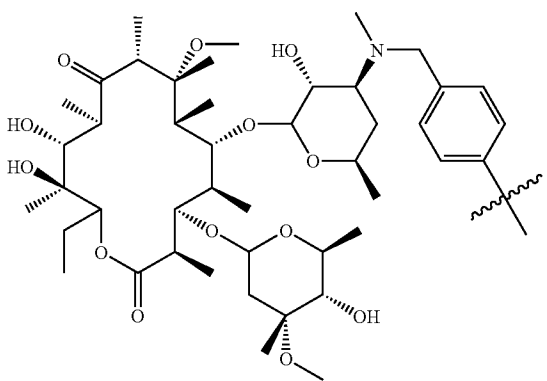
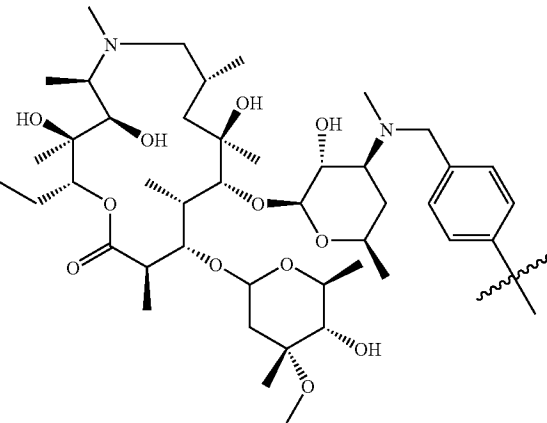
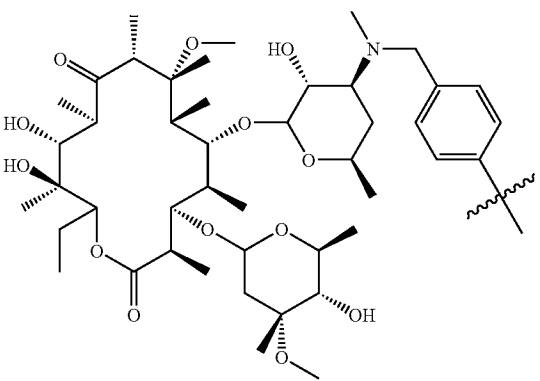
Antiparasitic activity of non-peptide HDAC inhibitors							
15		9	223.4	3.40	7.00	1.40	0.80
16		9	226.7	3.50	7.00	0.84	0.88
Chloroquine	—	NT	NT	NT	0.017	0.125	
Artemisin	—	NT	NT	NT	0.004	0.006	
Pentamidin	—	NT	0.90	1.70	NT	NT	
Amphotericine B	—	NT	0.18	0.32	NT	NT	
SAHA	—	65	22	50	0.25	0.47	
Compd.	R	Cytotoxicity (VERO)		S.I. D6		IC <sub>50</sub> (μg/ml) (W2)	
1		NC		>5.3		(>5.1)	

TABLE 8-continued

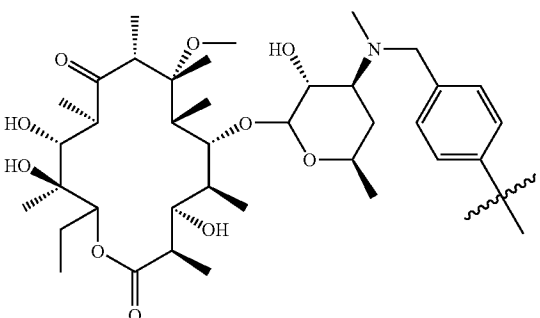
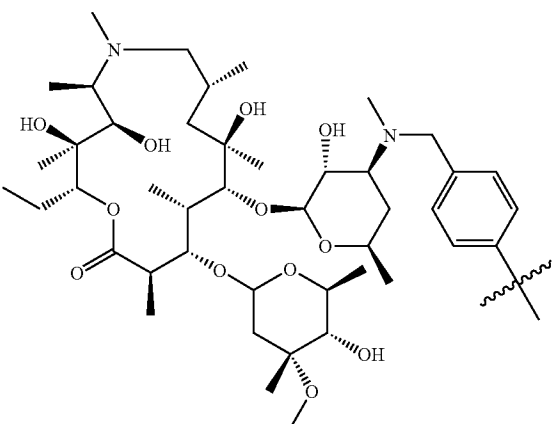
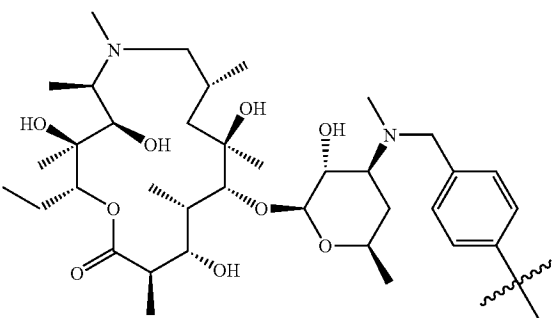
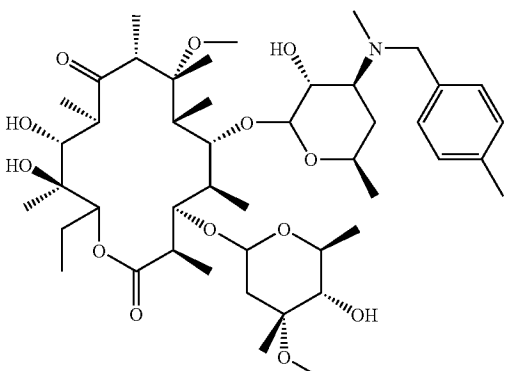
Antiparasitic activity of non-peptide HDAC inhibitors			
2		NC	>4.0 (>3.4)
3		NC	>4.0 (>3.0)
4		NC	>3.7 (>2.8)
5		NC	>17.6 (>15.4)

TABLE 8-continued

Antiparasitic activity of non-peptide HDAC inhibitors

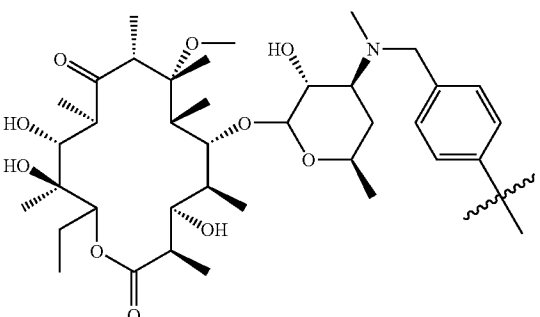
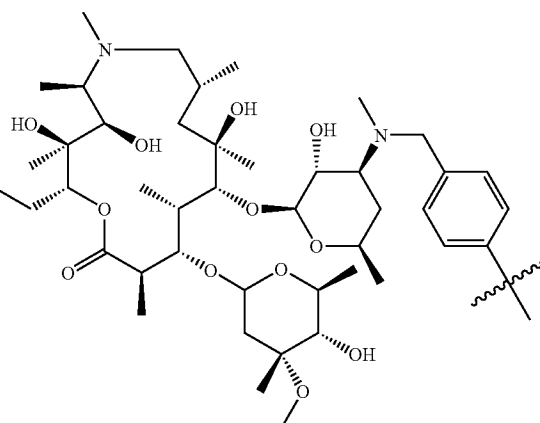
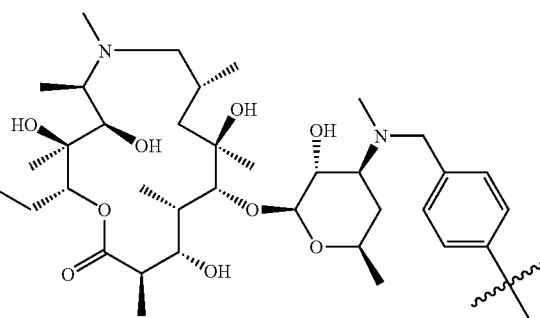
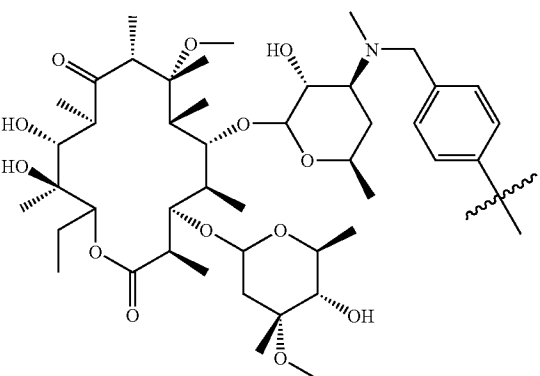
6		NC	>26.4 (>19.0)
7		NC	>20.7 (>47.6)
8		NC	>29.8 (28.0)
9		NC	>1.9 (>2.4)



TABLE 8-continued

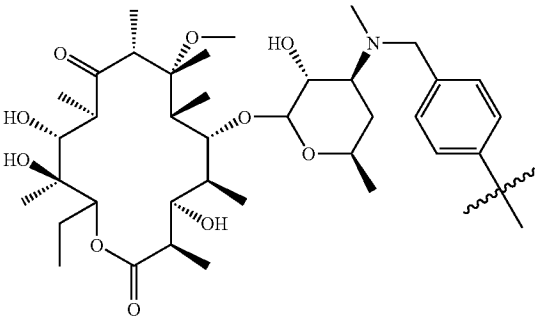
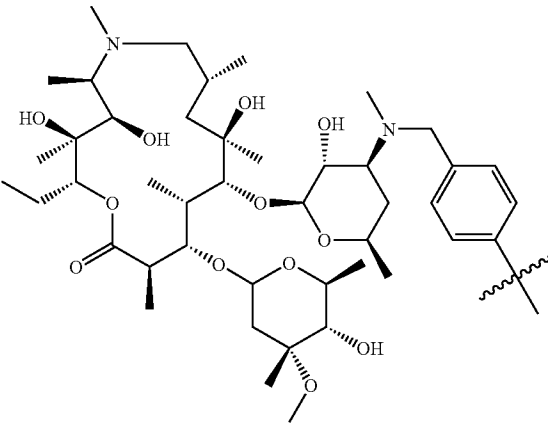
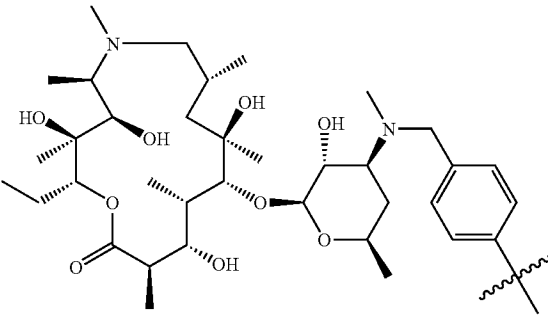
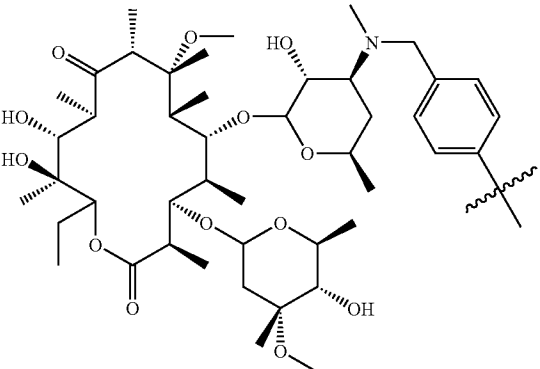
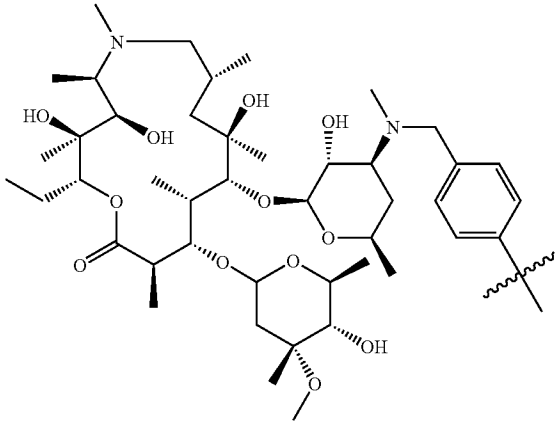
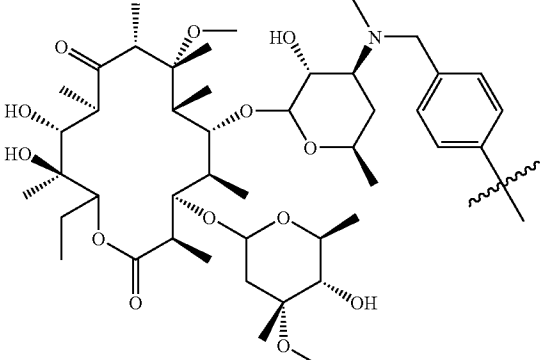
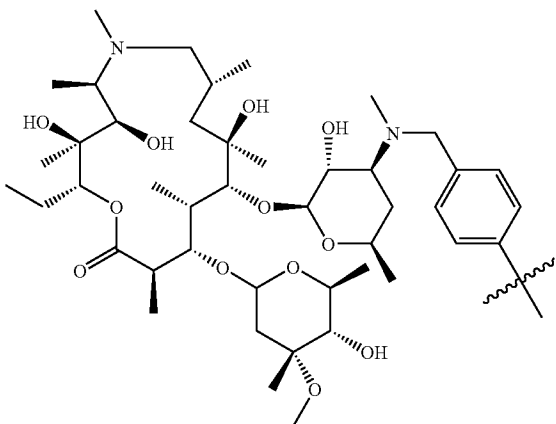
Antiparasitic activity of non-peptide HDAC inhibitors			
10		NV	>1.6 (>1.8)
11		NC	>2.0 (>2.1)
12		NC	>1.4 (>1.5)
13		NC	>2.8 (>4.8)

TABLE 8-continued

Antiparasitic activity of non-peptide HDAC inhibitors			
14		NC	>4.3 (<5.7)
15		NC	>3.4 (>6.0)
16		NC	>5.7 (>5.4)
	Chloroquine	NT	NT
	Artemisin	NT	NT
	Pentamidin	NT	NT
	Amphotericine B	NT	NT
	SAHA	1.20	4.8 (2.5)

NA = Not active up to the highest concentration tested

NC = Not cytotoxic up to the highest concentration tested

NT = Not tested

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The non-peptide macrocyclic HDAC inhibitors potently inhibited the proliferation of both the sensitive and resistant strains of *P. falciparum* with IC<sub>50</sub> ranging from 0.1 µg/mL to 3.5 µg/mL (Table 1). In particular, compounds 5-8 in Table 8, derived from either the 14- or 15-membered macrolide analogs macrolides and having 6 methylene spacers separating the triazole ring from the zinc-binding hydroxamic acid group (n=6), have the most potent antimalarial activities in this series. These compounds are equipotent or >4-fold more potent than the control compound SAHA. Moreover, they are several folds more selectively toxic to either strains of *P. falciparum* compared to SAHA. The antimalarial activities of these macrocyclic HDACi followed a similar trend as their anti-HDAC activity against HDAC 1/2 from HeLa nuclear extract, suggesting that parasite HDACs could be an intracellular target of these compounds.

Compounds 5 and 9 exhibited modest activity against the promastigote stage of *L. donovani*. This result is contrary to previous data on simple aryltriazolylhydroxamates which have antimalarial and antileishmanial activities that followed a similar trend.

A comparison of the antileishmanial activities of compounds 13 and 14, analogs with n=8, revealed an interesting disparity between the activity of 14- and 15-membered macrocyclic rings. 14-Membered compound 13 is 5- to 6-fold more potent than its 15-membered congener 14. However, this disparity dissipates after a single methylene group extension (n=9), as compounds 15 and 16 have virtually indistinguishable antileishmanial activities. Comparatively, compounds 13, 15 and 16, analogs with the most potent antileishmanial activities, are about 7- to 10-fold more potent than SAHA and approximately 3-fold less potent than pentamidine.

Since these non-peptide macrocyclic HDACi have nanomolar anti-HDAC activities, the observed disparity in the trend of their antimalarial and antileishmanial activities may have implication in the organization of the active sites of the relevant *P. falciparum* and *L. donovani* HDAC isozymes. These observations provide additional evidence of the suitability of HDAC inhibition as a viable therapeutic option to curb infections caused by apicomplexan protozoans and trypanosomatids (1, 5, 6, 14) and could facilitate the identification of other HDACi that are more selective for either parasite.

The antiparasitic activity of ketolides 56 were also evaluated against *P. falciparum* and *L. donovani*. The results are shown in Table 9.

TABLE 9

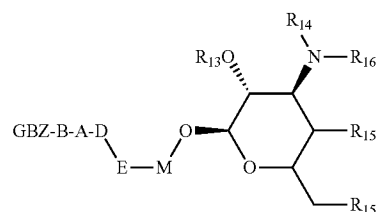
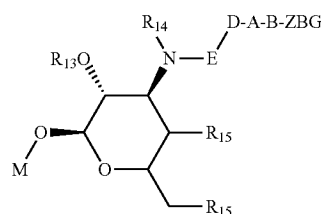
Antiparasitic activity of ketolide non-peptide HDAC inhibitors							
		Antimalarial Activity					
		Antileishmanial Activity	<i>Plasmodium falciparum</i>	<i>Plasmodium falciparum</i>	Cytotoxicity (VERO)	S.I.	
Compound	n	IC <sub>50</sub> (µg/ml)	IC <sub>90</sub> (µg/ml)	(D6 clone) IC <sub>50</sub> (µg/ml)	(W2 clone) IC <sub>50</sub> (µg/ml)	IC <sub>50</sub> (µg/ml) <sup>a</sup>	D6 (W2)
56a	1	NA	NA	0.96	1.80	NC	>5.0 (no value)
56b	2	NA	NA	0.14	0.12	NC	>34 (>39.7)
56c	3	19	36	1.20	1.50	NC	>4.0 (>3.2)
56d	4	14	32	1.20	1.30	NC	>4.0 (>3.7)
56e	5	4.8	23	1.20	0.84	NC	>4.0 (>5.7)
Chloroquine	—	NT	NT	0.011	0.14	NT	NT
Artemisinin	—	NT	NT	0.006	0.009	NT	NT
Pentamidine	—	1.4	6.0	NT	NT	NT	NT
Amphotericine B	—	0.08	0.3	NT	NT	NT	NT
SAHA	—	22	50	0.25	0.47	1.20	4.8 (2.5)

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Compounds 56a-e potently inhibit the proliferation of both the sensitive and resistant strains of *P. falciparum* with IC<sub>50</sub> ranging from 0.12 µg/mL to 1.5 µg/mL (Table 9). 56b has the most potent antimalarial activity which is between 2- to 4-fold more potent than the control compound SAHA. Moreover, 56b is several folds more selectively toxic to either strains of *P. falciparum* compared to SAHA. Compounds 56c-e have a moderate to good antileishmanial activity against the promastigote stage of *L. donovani* activity which peaks with 56e, the analog with the longest methylene spacers in the series. This result is in contrast to the antimalarial activity that mirrors compound anti-HDAC activity and it may have implications in the organization of the active sites of the relevant *P. falciparum* and *L. donovani* HDAC isozymes. We have observed a similar methylene spacer-length dependence in the antileishmanial activity of macrocyclic HDACi 14- and 15-membered macrolides as shown in Table 8. The foregoing observations further support the suitability of HDAC inhibition as a viable therapeutic strategy to curb infections caused by apicomplexan protozoans and trypanosomatids.

I claim:

1. A compound of Formula I or II:



wherein M represents a macrolide subunit,

E is a C<sub>1-6</sub> group, optionally containing one or more heteroatoms, wherein the carbon atoms and/or heteroatoms are in a linear and/or cyclic arrangement,

D is a substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl group,

A is a linking group connected to D,

B is an alkyl, heteroalkyl, alkylaryl or alkylheteroaryl spacer group,

ZBG is a Zinc Binding Group,

R<sub>13</sub> is selected from hydrogen, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoyl group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group,

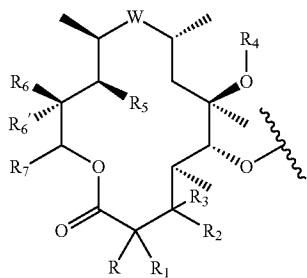
R<sub>14</sub> and R<sub>16</sub> is selected from hydrogen, hydroxyl, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoyl group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group,

R<sub>15</sub> is hydrogen or —OR<sub>17</sub>,

R<sub>17</sub> is selected from a group consisting of hydrogen, a substituted or unsubstituted C<sub>1-6</sub> alkyl group, a substituted or unsubstituted C<sub>2-6</sub> alkenyl group, a substituted or unsubstituted C<sub>2-6</sub> alkynyl group, a substituted or unsubstituted C<sub>1-6</sub> alkanoyl group, a substituted or unsubstituted C<sub>2-6</sub> carbamate group, a substituted or unsubstituted C<sub>2-6</sub> carbonate group, or a substituted or unsubstituted C<sub>2-6</sub> thiocarbamate group.

2. The compound of claim 1, wherein the macrolide subunit is a multi-member lactonic ring structure.

3. The compound of claim 2, wherein the macrolide subunit has the structure:



wherein

W is selected from —C(O)—, —C(=NR<sub>11</sub>)—, —CH(—OR<sub>11</sub>)—, —NR<sub>11</sub>CH<sub>2</sub>—, —CH<sub>2</sub>NR<sub>11</sub>—, —CH(NR<sub>11</sub>R<sub>11</sub>)—, —C(=NNR<sub>11</sub>R<sub>11</sub>)—, —NR<sub>11</sub>C(O)—, —C(O)NR<sub>11</sub>—, and —C(=NR<sub>11</sub>)—;

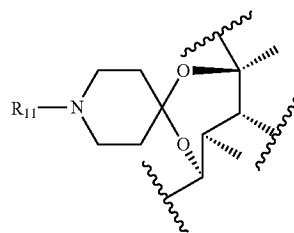
R is selected from the group consisting of H and C<sub>1-6</sub> alkyl;

R<sub>1</sub> is selected from the group H, halogen, —NR<sub>11</sub>R<sub>11</sub>, —NR<sub>11</sub>C(O)R<sub>11</sub>, —OR<sub>11</sub>, —OC(O)R<sub>11</sub>, —OC(O)OR<sub>11</sub>, —OC(O)NR<sub>11</sub>R<sub>11</sub>, —OC(O)C<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)C<sub>1-6</sub> alkenyl-R<sub>12</sub>, —OC(O)C<sub>1-6</sub> alkynyl-R<sub>12</sub>, —OC(O)OC<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)NR<sub>11</sub>C<sub>1-6</sub> alkyl-R<sub>12</sub>, C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkenyl, C<sub>1-6</sub> alkynyl, optionally is substituted with one or more R<sub>12</sub> groups;

R<sub>2</sub> is H;

R<sub>3</sub> is selected from H, —OR<sub>11</sub>, —OC<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)R<sub>11</sub>, —OC(O)C<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)OR<sub>11</sub>, —OC(O)OC<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)NR<sub>11</sub>R<sub>11</sub>, —OC(O)NR<sub>11</sub>C<sub>1-6</sub> alkyl-R<sub>12</sub>; alternatively, R<sub>3</sub> is a pyran ring which can be substituted as defined above in Formulae I and II;

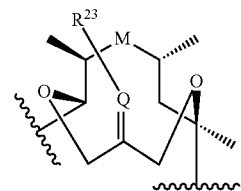
R<sub>4</sub> is selected from H, R<sub>11</sub>, —C(O)R<sub>11</sub>, —C(O)OR<sub>11</sub>, —C(O)NR<sub>11</sub>R<sub>11</sub>, —C<sub>1-6</sub> alkyl-T-R<sub>11</sub>, —C<sub>2-6</sub> alkenyl-T-R<sub>11</sub>, and —C<sub>2-6</sub> alkynyl-T-R<sub>11</sub>; alternatively R<sub>3</sub> and R<sub>4</sub> taken together form



T is selected from —C(O)—, —C(O)O—, —C(O)NR<sub>11</sub>—, —C(=NR<sub>11</sub>)O—, —C(=NR<sub>11</sub>)NR<sub>11</sub>—, —OC(O)—, —OC(O)O—, —OC(O)NR<sub>11</sub>—, —NR<sub>11</sub>C(O)—, —NR<sub>11</sub>C(O)O—, —NR<sub>11</sub>C(O)NR<sub>11</sub>—, and —S(O)<sub>p</sub>—, wherein p is 0-2;

R<sub>5</sub> is selected from R<sub>11</sub>, —OR<sub>11</sub>, —NR<sub>11</sub>R<sub>11</sub>, —OC<sub>1-6</sub> alkyl-R<sub>12</sub>, —C(O)R<sub>11</sub>, —C(O)C<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)R<sub>11</sub>, —OC(O)G<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)OR<sub>11</sub>, —OC(O)OC<sub>1-6</sub> alkyl-R<sub>12</sub>, —OC(O)NR<sub>11</sub>R<sub>11</sub>, —OC(O)NR<sub>11</sub>C<sub>1-6</sub> alkyl-R<sub>12</sub>, —C(O)C<sub>2-6</sub> alkenyl-R<sub>12</sub>, and —C(O)C<sub>2-6</sub> alkynyl-R<sub>12</sub>;

alternatively, R<sub>4</sub> and R<sub>5</sub> taken together with the atoms to which they are bonded, form:



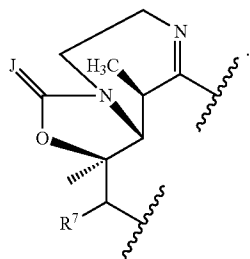
wherein, Q is CH or N, and R<sub>23</sub> is —OR<sub>11</sub> or R<sub>11</sub>;

R<sub>6</sub> is selected from —OR<sub>11</sub>, —C<sub>1-6</sub> alkoxy-R<sub>12</sub>, —C(O)R<sub>11</sub>, —OC(O)R<sub>11</sub>, —OC(O)OR<sub>11</sub>, —OC(O)NR<sub>11</sub>R<sub>11</sub>, —NR<sub>11</sub>R<sub>11</sub>;

alternatively, R<sub>5</sub> and R<sub>6</sub> taken together with the atoms to which they are attached form a 5-membered ring by attachment to each other through a linker selected from —OC(R<sub>12</sub>)<sub>2</sub>O—, —OC(O)O—, —OC(O)NR<sub>11</sub>—, —NR<sub>11</sub>C(O)O—, —OC(O)NR<sub>11</sub>—, —NR<sub>11</sub>C(O)O—, —OC(O)NNR<sub>11</sub>R<sub>11</sub>—, —NR<sub>11</sub>R<sub>11</sub>C(O)O—, —OC(O)C(R<sub>12</sub>)<sub>2</sub>—, —C(R<sub>12</sub>)<sub>2</sub>C(O)O—, —OC(S)O—, —OC(S)NR<sub>11</sub>—, —NR<sub>11</sub>C(S)O—, —OC(S)NR<sub>11</sub>—, —NR<sub>11</sub>C(S)O—, —OC(S)NNR<sub>11</sub>R<sub>11</sub>—, —NR<sub>11</sub>R<sub>11</sub>C(S)O—, —OC(S)C(R<sub>12</sub>)<sub>2</sub>—, —C(R<sub>12</sub>)<sub>2</sub>C(S)O—;

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alternatively, W, R<sub>5</sub>, and R<sub>6</sub> taken together with the atoms to which they are attached form:



wherein J is selected from the group consisting of O, S, and NR<sub>11</sub>;

R<sub>6</sub> is selected from H, unsubstituted or substituted C<sub>1-4</sub> alkyl, C<sub>2-4</sub> alkenyl, which can be further substituted with C<sub>1-2</sub> alkyl or one or more halogens, C<sub>2-4</sub> alkynyl, which can be further substituted with C<sub>1-2</sub> alkyl or one or more halogens, aryl or heteroaryl, which can be further substituted with C<sub>1-2</sub> alkyl or one or more halogens, —C(O)H, —COOH, —CN, —COOR<sub>11</sub>, —C(O)NR<sub>11</sub>R<sub>11</sub>, —C(O)R<sub>11</sub>, —C(O)SR<sub>11</sub>;

alternatively R<sub>6</sub> and R<sub>6</sub>, taken together with the atom to which they are attached to form an epoxide, a carbonyl, an olefin, or a substituted olefin, or a C<sub>3-7</sub> carbocyclic, carbonate, or carbamate, wherein the nitrogen of the carbamate can be further substituted with a C<sub>1-6</sub> alkyl; R<sub>7</sub> is selected from C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, and C<sub>2-6</sub> alkynyl, optionally is substituted with one or more R<sub>12</sub> groups;

R<sub>11</sub>, for each occurrence, is selected from H, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>6-10</sub> saturated, unsaturated, or aromatic carbocycle, 3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from nitrogen, oxygen, and sulfur, —C(O)—C<sub>1-6</sub> alkyl, —C(O)—C<sub>2-6</sub> alkenyl, —C(O)—C<sub>2-6</sub> alkynyl, —C(O)—C<sub>6-10</sub> saturated, unsaturated or aromatic carbocycle, —C(O)—3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from nitrogen, oxygen, and sulfur, —C(O)O—C<sub>1-6</sub> alkyl, —C(O)O—C<sub>2-6</sub> alkenyl, —C(O)O—C<sub>2-6</sub> alkynyl, —C(O)O—C<sub>6-10</sub> saturated, unsaturated, or aromatic carbocycle, —C(O)O—3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur, and —C(O)NR<sub>13</sub>R<sub>13</sub>, optionally is substituted with one or more R<sub>12</sub> groups; and

R<sub>12</sub> is selected from R<sub>14</sub>, C<sub>1-8</sub> alkyl, C<sub>2-8</sub> alkenyl, C<sub>2-8</sub> alkynyl, C<sub>3-12</sub> saturated, unsaturated, or aromatic carbocycle, 3-12 membered saturated, unsaturated, or aromatic heterocycle containing one or more heteroatoms selected from nitrogen, oxygen, and sulfur, optionally substituted with one or more substituents.

4. The compound of claim 1, wherein D is a phenyl, biphenyl, or naphthyl group.

5. The compound of claim 1, wherein A is an amid; —O—, or 1,2,3-triazolyl group.

6. The compound claim 1, wherein B is an alkyl group having from 1-12 carbon atoms.

7. The compound claim 1, wherein the zinc binding group is selected from the group consisting of hydroxamate, N-formyl hydroxylamine, pyridine thione, thiol, benzamide, and hydroxyl pyridone.

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8. The compound claim 1 selected from the group consisting of the compounds in Table 3.

9. The compound of claim 8, wherein the compound is an azithromycin-arylalkyltriazolyl hydroxamate, a tricyclic ketolide-arylalkyltriazolyl hydroxamate or a clarithromycin-arylalkyltriazolyl hydroxamate.

10. The compound of claim 8, wherein the compound is a descladinoseazithromycin-arylalkyltriazolyl hydroxamate or a descladinoseclarithromycin-arylalkyltriazolyl hydroxamate.

11. A pharmaceutical composition comprising an effective amount of the compound of claim 1 in combination with a pharmaceutically acceptable diluent, excipient, or carrier.

12. The composition of claim 11, wherein the composition is administered enterally.

13. The composition of claim 11, wherein the composition is administered parenterally.

14. The composition of claim 11, wherein the composition is formulated for immediate release, modified release, and combinations thereof.

15. The composition of claim 14, wherein the formulation is selected from the group consisting of delayed release, extended release, pulsatile release, and combinations thereof.

16. A method of treating a disease or disorder in a human or animal comprising administering an effective amount of a compound claim 1, wherein the disease or disorder to be treated is selected from the group consisting of cancer and parasitic infections.

17. The method of claim 16, wherein the cancer is selected from the group consisting of lung cancer, myeloma, leukemia, lymphoma, breast cancer, prostate cancer, pancreatic cancer, cervical cancer, ovarian cancer, and liver cancer.

18. The method of claim 16, wherein the parasitic infection is selected from the group consisting of malaria and leishmaniasis.

19. The method of claim 16 wherein the compound is administered enterally.

20. The method of claim 19, wherein the compound is formulated in a solid oral dosage form selected from the group consisting of tablets, capsules, dragees, and caplets.

21. The method of claim 19, wherein the compound is formulated in a liquid oral dosage form selected from the group consisting of solutions, suspensions, and syrups.

22. The method of claim 16, wherein the compound is administered parenterally.

23. The method of claim 22, wherein the formulation is a suspension or solution.

24. The compound of claim 8, wherein the compound is an azithromycin-arylalkyltriazolyl hydroxypyridinone, a descladinoseazithromycin-arylalkyltriazolyl hydroxypyridinone, a clarithromycin-arylalkyltriazolyl hydroxypyridinone, a tricyclic ketolide-arylalkyltriazolyl hydroxypyridinone, or a descladinoseclarithromycin-arylalkyltriazolyl hydroxypyridinone.

25. The compound of claim 8, wherein the compound is an azithromycin-arylalkyltriazolyl hydroxypyridinethione, a descladinoseazithromycin-arylalkyltriazolyl hydroxypyridinethione, a clarithromycin-arylalkyltriazolyl hydroxypyridinethione, a tricyclic ketolide-arylalkyltriazolyl hydroxypyridinethione, or a descladinoseclarithromycin-arylalkyltriazolyl hydroxypyridinethione.

26. The compound of claim 8, wherein the compound is an azithromycin-arylalkyltriazolyl benzamide, a descladinoseazithromycin-arylalkyltriazolyl benzamide, a clarithromycin-arylalkyltriazolyl benzamide, a tricyclic ketolide-arylalkyltriazolyl benzamide, or a descladinoseclarithromycin-arylalkyltriazolyl benzamide.

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